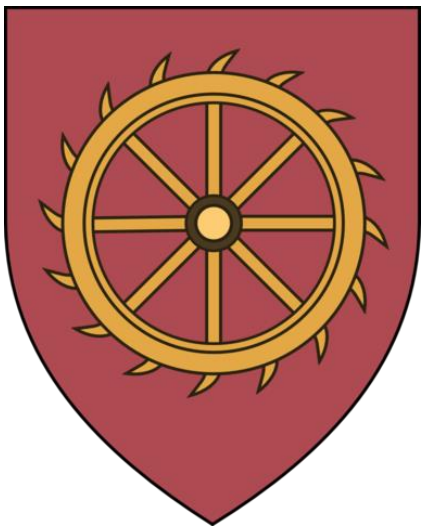
“Is Butter a Carb?”

Neural mechanisms of nutrient-sensing and food reward in the human brain



Putu Agus Khorisantono

St Catharine’s College

Department of Physiology, Development and Neuroscience

University of Cambridge

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This dissertation is submitted for the Degree of Doctor of Philosophy.

Personal Declaration

This thesis was completed in the department of Physiology, Development and Neuroscience under the supervision of Dr Fabian Grabenhorst.

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text. I further state that no substantial part of my thesis has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. It does not exceed the prescribed word limit for the Biology Degree Committee.

Summary

Putu Agus Khorisantono

"Is Butter a Carb?" Neural mechanisms of nutrient-sensing and food reward in the human brain

Sensing the nutrient composition of a food and the processing of this information by the brain's reward system to regulate food consumption are crucial biological needs. However, dysfunction in neural reward pathways may also lead to overconsumption of certain nutrients, contributing to obesity and comorbid diseases. In the context of fat, the oral sensory mechanism of its detection is disputed, although there is substantial evidence for fat detection through oral textural properties. In this thesis, I investigate the neural correlates related to the specific textural properties of oral food stimuli with defined nutrient contents, as well as their formally measured economic reward values and psychophysical ratings during functional Magnetic Resonance Imaging (fMRI) in healthy human volunteers. These results are then correlated with an *ad-libitum* naturalistic eating test.

The thesis contains the following chapters: Chapter I discusses the key background literature; Chapter II focuses on the optimisation of the design and stimuli; Chapter III provides a detailed analysis of behavioural data, through basic psychophysical ratings of food stimuli and modelling of subjective value data; Chapter IV describes the results of the neuroimaging component of the experiment, and Chapter V discusses the results of the project in the context of current literature.

This project investigates the textural contributions to sensory fat detection and reward valuation. Crucially, it is the first time a formal fMRI investigation is done on the oral-lubricative nature of fat, demonstrating encoding of sliding friction in the midposterior insula and the oral somatosensory cortex, which supports the concept that fat detection occurs through texture. Furthermore, our results highlight the unique role of the orbitofrontal cortex in processing food texture parameters, their subjective perception, and integration to subjective value, before subsequent evaluation in the ventromedial prefrontal cortex.

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List of Common Abbreviations

Here we list the common abbreviations used throughout the thesis:

- **ACC** Anterior Cingulate Cortex
- **Angular Gyrus** Angular Gyrus
- **ANOVA** Analysis of Variance
- **BDM** Becker-DeGroot-Marschak
- **CMC** Carboxymethyl cellulose
- **CSF** Coefficient of Sliding Friction
- **dlPFC** Dorsolateral Prefrontal Cortex
- **fMRI** Functional Magnetic Resonance Imaging
- **FWE** Family-wise Error
- **GLM** General Linear Model
- **HFHS** High-Fat High-Sugar
- **HFLS** High-Fat Low-Sugar
- **LFHS** Low-Fat High-Sugar
- **LFLS** Low-Fat Low-Sugar
- **MNI** Montreal Neurological Institute
- **MVPA** Multivariate Pattern Analysis
- **OFC** Orbitofrontal Cortex
- **Oral SSC** Oral Somatosensory Cortex
- **pACC** Pregenual Anterior Cingulate Cortex
- **PDMS** Poly Dimethyl Siloxane
- **PFC** Prefrontal Cortex
- **Post** Posterior
- **PPI** Psychophysiological Interaction
- **Prim** Primary
- **Sec Visual** Secondary Visual Area
- **SI** Primary Somatosensory Cortex
- **Supp Motor** Supplementary Motor Area
- **Supramarg** Supramarginal
- **SVC** Small Volume Correction
- **SVM** Support Vector Machine
- **Temp Gyrus** Temporal Gyrus
- **TRF** Translational Research Facility
- **Visuomotor Area** Visuo-motor Area
- **VPM** Medial Ventral Posterior Nucleus
- **VPMpc** Parvocellular Medial Ventral Posterior Nucleus
- **vmPFC** Ventromedial Prefrontal Cortex
- **WTP** Willingness to Pay

Chapter I – Introduction

1.1 – Background

Food consumption is a basic biological need. In order to ensure survival and fuel various basic activities, organisms require a variety of macro- and micro-nutrients. Energy is derived from the consumption of a relatively large amount of macronutrients, which can be broadly categorised into fats, carbohydrates and proteins (Lloyd, McDonald, & Crampton, 1978). In order to regulate the consumption of these macronutrients, the brain's reward system responds to them in such a manner that eating becomes a pleasurable act (E. T. Rolls, 2011). However, this rewarding aspect of eating may also lead to overconsumption of some, or all, macronutrients which would cause an energy imbalance, which has been implicated in obesity and other co-morbid diseases (Alonso-Alonso et al., 2015). For example, patients with a predisposition towards obesity, such as those with Melanocortin-4 receptor mutations, display a different neural response to food cues in areas such as the striatum (van der Klaauw et al., 2014), which implies that the differences in the reward processing of food lead to variation in eating behaviour. However, the specific mechanisms in the human brain that underlie reward-guided eating behaviour, especially the consumption of specific nutrients, are still largely unknown. Greater understanding of these underlying mechanisms would shed light on why individuals may have different eating behaviours and, therefore, why some are prone to overconsumption and obesity.

Planning of food consumption in the longer term would require learning the nutrient contents of various foods in order to optimise future meal compositions. Reward structures in the brain have been known to respond to different sensory properties of rewards (E. T. Rolls, 2011). In the case of food, activation of these structures has been linked to sensory food qualities, ranging between visual properties (food cues, shape or colour), smell, taste (such as sweetness) and oral texture (smooth or viscous). These properties that are sensed just before and during ingestion tell the organism about the nutrient components of the food.

In a typical day, one makes hundreds of food- and beverage-related decisions, despite only being completely aware of a fraction of them. Ultimately, these decisions come down to choosing to consume certain foods over either other available options or nothing at all. These choices are based on subjective preferences, which have been

studied in both economics (Samuelson, 1983) and psychology (Kahneman & Tversky, 2000). Each individual option is assigned a value, and the choices are made according to a value comparison (Padoa-Schioppa, Jandolo, & Visalberghi, 2006). These values are represented in reward processing regions such as the orbitofrontal cortex (OFC; Padoa-Schioppa & Assad, 2008) and the amygdala (Schultz, 2015). Within the context of this thesis, subjective value is defined as the importance placed on a good by an individual, as seen through choice tasks (Padoa-Schioppa & Assad, 2006) or formal economic auctions (Becker, Degroot, & Marschak, 1964), whereas reward value refers to a more general valuation of the good observed through tasks such as pleasantness rating scales (Grabenhorst, Rolls, Parris, & d'Souza, 2010). The initial sensory analysis of the food leads to a reward valuation of the specific components of the food, which is then integrated as a reward value for the food itself. However, the brain's responses in the reward areas to orally sensed food stimuli have not been studied in detail, as current imaging studies on food choice have largely been based on visual stimuli (DiFeliceantonio et al., 2018; Suzuki, Cross, & O'Doherty, 2017), thereby leading to a lack of translatability to real-life situations that affect eating behaviour.

In this thesis, I address these issues using functional magnetic resonance imaging (fMRI) in healthy human volunteers to explore basic hypotheses about the function of the reward areas in food valuations and choices, combining neuroimaging with detailed psychophysical tests and a naturalistic eating test. The thesis reviews the study design, stimulus design, fMRI project and behavioural testing, identifying how the results of each segment help elucidate the neural reward mechanisms in food choice. This introductory chapter will first consider the sources of food reward values, specifically nutrient and sensory food properties. The anatomy and function of specific brain areas implicated in food reward will then be discussed, as a basis for interpreting the experimental findings discussed in subsequent chapters.

1.2 – Nutrients and Sensory Food Properties

1.2.1 – Nutrients

1.2.1.1 – Carbohydrates

Carbohydrates are a basic type of macronutrient, consisting of carbon, hydrogen and oxygen molecules and being categorised into glycaemic and non-glycaemic carbohydrates (Lloyd et al., 1978). This distinction refers to the body's ability to use the

carbohydrate for metabolism. Glycaemic carbohydrates can be digested and subsequently absorbed to provide nutrition for cells in the form of carbohydrates. On the other hand, carbohydrates that cannot be digested in such a manner and therefore do not enter the body through the small intestines, such as cellulose, pectins and retrograded amylose, are classified as non-glycaemic carbohydrates as they are not utilised in cell metabolism. However, non-glycaemic carbohydrates are still crucial to a healthy diet as they provide dietary fibre or roughage that assist the peristaltic movement of food along the gastrointestinal tract in addition to providing substrates for colonic microflora (Sandstrom et al., 2012).

Typically, the function of glycaemic carbohydrates in the human body is to provide glucose that is subsequently metabolised for energy in human cells, providing around 4 kcal/g. Simple carbohydrates, like fructose and glucose, consist of one and two sugar units respectively and are consequently classified as monosaccharides and disaccharides. The main source of simple carbohydrates in a typical Western diet would be fruits, berries, juices, soft drinks and sweets. Polysaccharides such as starch consist of long chains of sugars that form a macromolecule, and are thus termed complex carbohydrates, which are typically present in the Western diet in the form of bread, cereals and potatoes. Through typically enzymatic reactions with enzymes such as amylase in the saliva, complex carbohydrates such as starch are hydrolysed into simple carbohydrates. This process occurs as early as during the mastication period in the mouth (Douglas, 1994), which allows humans to sense carbohydrates and sugars upon ingestion with ease due to the presence of sugar-sensing taste receptors on the tongue.

As carbohydrates provide the main source of glucose for cell metabolism, they should form a significant portion of the energy intake in a healthy diet, with many health organisations recommending around 45-60% of one's daily energy consumption. However, due to the strong links between high levels of free sugar intake and incidence of diseases such as type 2 diabetes (Sonestedt, Øverby, Laaksonen, & Eva Birgisdottir, 2012), this recommendation also contains a caveat whereby the daily energy intake from simple carbohydrates should not exceed 10% of the daily energy consumption (World Health Organization, 2019). Due to the key role carbohydrates play in metabolism, they are particularly relevant for our project on food reward, as they form a large proportion of the macronutrients consumed in a typical diet (Stubbs, Van Wyk, Johnstone, & Harbron,

1996). The nature of this project focuses on neural and behavioural responses during oral food sensing, such that monosaccharides and disaccharides such as lactose and glucose would be best candidate carbohydrates for the project.

1.2.1.2 – Fats

In addition to carbohydrates, fats are also classified as a macronutrient. Fats are ester compounds of fatty acids and, in the context of macronutrients, typically refer to triglycerides (Jones & Schoeller, 1988). Fatty foods in general contain essential fatty acids that cannot be produced by the body, such as linoleic acid and alpha-linoleic acid and therefore need to be ingested (Sandstrom et al., 2012). Furthermore, fat plays a pivotal structural role in the body, such as forming cell membranes and providing structural support in adipose tissue to cushion internal organs (Batra & Siegmund, 2012). This is in addition to forming glial cells and the myelin sheath of neurons, thereby proving crucial to the survival of the organism (Nave, Tzvetanova, & Schirmeier, 2017). In terms of dietary functions, the liquid state of fat at body temperature makes it a non-polar solvent, which allows it to transport various nutrients required by the body that are not soluble in water, such as vitamins A, D, E and K (Sandstrom et al., 2012).

Adipose tissues in which fat is stored also play various vital functions in hormonal balance. One example of such a function is the releasing of leptin which interacts with the hypothalamus in the brain to signal satiety levels (Elmquist, Ahima, Elias, Flier, & Saper, 1998; Elmquist, Ahima, Maratos-Flier, Flier, & Saper, 1997). Leptin sensitivity is strongly linked with body weight and body fat composition, as patients who are leptin resistant consume more calories and have a greater propensity for weight gain (Kishi & Elmquist, 2005; Montague et al., 1997). In addition, in various animal models, knocking out leptin receptors leads to hyperphagic and obese animals in comparison to the wild type animals (Halaas et al., 1995). Therefore, there is a close and complex relationship between leptin and body-fat percentage, which is also determined in part by dietary fat (Skorupa, Dervisefendic, Zwiener, & Pletcher, 2008).

Fats provide around 9 kcal/g of energy (Sandstrom et al., 2012). This relatively high caloric density makes them more advantageous in the context of the survival of the organism in times of low caloric availability. The storage of excess energy as fat in adipose tissue also provides many organisms with the ability to survive relatively long periods of low caloric intake. However, in environments where there is more abundance of food

sources, the ready availability and palatability of highly caloric foods – primarily driven by the high fat and carbohydrate content of the foods – may lead to an obesogenic environment that encourages higher daily calorie consumption (Brunstrom, Drake, Forde, & Rogers, 2018). Furthermore, increased intake of certain types of fat, such as saturated fatty acids, has been strongly linked to increased risks of heart disease and stroke owing to its link to levels of low-density lipoproteins in the bloodstream (Kirkhus et al., 2015). In light of these factors, various governing bodies in nutrition suggest that fat intake be limited, such as the suggestion of 25-40% of a person's daily energy intake by the Nordic Nutrition Recommendations (Sandstrom et al., 2012). Given the large percentage of the average daily calories coming from fat, and the propensity for overconsumption of fatty foods (World Health Organization, 2019), dietary fat consumption appears to be particularly relevant to food reward value and, therefore, will be one of the main foci of this thesis. In this project, fat content of the stimuli will be modulated using dairy cream, which is added to liquid food rewards that are delivered to participants similarly to previous neuroimaging studies (Grabenhorst & Rolls, 2014; Grabenhorst, Rolls, et al., 2010).

1.2.1.3 – Protein

In addition to carbohydrates and fat, protein also forms part of the macronutrients required in a healthy diet. Unlike fats and carbohydrates, amino acids contain nitrogen and can therefore be thought of as a different 'currency' than hydrocarbons (Lloyd et al., 1978). Similar to fats and carbohydrates, dietary protein can be metabolised as a source of energy for the body providing about 4kcal/g (Mann & Truswell, 2017; Sandstrom et al., 2012). However, protein also plays a more specific role in that it is a major constituent of all parts of the body, in addition to performing a host of biological functions ranging from enzymatic catalysis to hormonal functions. In general, the proteins pertinent to human bodily functions are built up from 20 basic building blocks known as amino acids, 9 of which are termed essential amino acids as they cannot be synthesised by the body and must be consumed (Mann & Truswell, 2017).

Although most of the protein used in these functions and integrated into body tissue is obtained from constant deconstruction of existent proteins and re-synthetisation of the resultant amino acids, a significant amount of nitrogen is excreted through urine as urea, creatinine and uric acid, as well as the skin, hair and nails.

Therefore, there is still a need to supplement a healthy diet with sufficient protein (Sandstrom et al., 2012). This is especially true in cases where the individual aims to increase muscle mass such as athletes, as the muscle fibres themselves are formed through the use of protein, such that the rate-limiting factor in terms of muscle growth for an anabolic cycle is the protein intake (Tarnopolsky et al., 1992; Tipton & Wolfe, 2004).

Dietary protein may also prove helpful in maintaining hypocaloric diets. One mechanism related to this is the link between protein consumption and satiety, as the greater satiety levels elicited by high-protein diets are well-documented (Eisenstein, Roberts, Dallal, & Saltzman, 2002; Lejeune, Westerterp, Adam, Luscombe-Marsh, & Westerterp-Plantenga, 2006; Stubbs et al., 1996; Veldhorst et al., 2008; Westerterp-Plantenga, 2003). This may be due to the specific role of protein in most body functions as well as it being a basic building block for tissue. Most animals seem to have at least a sense of how much dietary protein they consume, as behaviours wherein total food intake appears to be linked to the absolute amount of protein consumed apply across species including drosophila (Skorupa et al., 2008), rodents (Sørensen, Mayntz, Raubenheimer, & Simpson, 2008) and humans (Martinez-Cordero et al., 2012; Raubenheimer & Simpson, 2019; Simpson & Raubenheimer, 2005). Moreover, protein also aids individuals aiming to incorporate a hypocaloric diet due to the higher metabolic cost involved in its processing in comparison to other macronutrients, known as the thermic effect of food (Crovetti, Porrini, Santangelo, & Testolin, 1998). For these reasons, it is sensible to encourage higher protein intake in the population, as this may help reduce the propensity for individuals to eat above their daily energy expenditure. The reference nutrient intake of protein is set at 0.75g of protein per kilogram bodyweight per day as set by the British Nutrition Foundation, whereas it is at 0.8g per kilogram of bodyweight as set by the Food and Nutrition Board of the USA (Volpi et al., 2013). These values are aimed at maintaining the nitrogen balance of the body by replacing excreted nitrogen. However, there is increasing evidence that a higher protein intake would be beneficial for the elderly (Bauer et al., 2013; Deutz et al., 2014), athletes (Tarnopolsky et al., 1992; Tipton & Wolfe, 2004) and those aiming to reduce their caloric intake (Westerterp-Plantenga, 2003). Despite this importance, protein is still regularly under-consumed in the general population (Bauer et al., 2013; Lloyd et al., 1978), such that protein content may not truly elicit the strong reward responses that seem to contribute to the prevalent overconsumption of

carbohydrates and fats. Therefore, in this thesis, the role of protein will be more to provide a control macronutrient instead of being the main focus.

1.2.2 – Sensory information about nutrients

The act of eating food is a complex sensory process that involves all sensory modalities. We are able to obtain the identity as well as the nutrient content of foods through the various sensory information provided surrounding the ingestion of said foods. When food is ingested, it activates a host of sensory processes associated with it. In order to assess if and how the food being consumed is nutritionally beneficial, there exist several pathways that inform the brain of the contents of the food. This section will cover the principal sensory modalities that are involved in the consumption of food and the nutrient detection mechanisms that lie therein, including the visual, olfactory, taste and mechanosensory properties that allow us to detect the nutrient content of foods.

1.2.2.1 – Visual Stimuli

In the moments leading up to ingestion, the food is represented by a visual cue, which may already have associations if the food has in the past been ingested. For example, one is able to identify an apple from a distance by its general shape and colour, the visual pattern of which would likely have been represented before in previous instances of apple consumption. On the other hand, a fruit one is unfamiliar with, for example a durian, would not have had its visual representations associated with any instances of consumption in the past. Furthermore, one would not be able to predict the metabolic effects involved in the ingestion of the durian, as its nutrient contents are unknown, whereas the apple is a familiar enough sensation that one is able to predict its macronutrient contents much more reliably. Therefore, the visual cues pertaining to a food play an important role in informing us of its nutrient contents.

The visual stimuli associated with a certain food item modify our perception when ingesting it. For example, the presence and intensity of a red colour in a liquid influences the perceived sweetness thereof (J. Johnson & Clydesdale, 1982), whereas a red container elicits greater perceived carbonation over that of a black receptacle (Mielby et al., 2018). In addition to modulating the subjective perception of both the taste and textural properties of foods, visual cues associated with previous consumption of a food may also affect preference of a food. Participants who were given a coloured drink with maltodextrin (a tasteless but caloric solute) and an equally tasteless coloured solution

showed greater preference for the colour associated with the calorically loaded drink, although they were unable to detect the difference between a maltodextrin-loaded drink and a control coloured solution (de Araujo, Lin, Veldhuizen, & Small, 2013). The postprandial effects associated with visual cues are therefore also crucial in determining the nutrient content of foods that are repeatedly consumed.

However, visual stimuli are not only used in the prior to the ingestion of a food but also serve as a manner in which we are able to estimate the amount of certain nutrients we have consumed, including the calories therein. This idea was of such importance to the inventor Nikola Tesla that he famously liked to eat only foods which he could visually judge in size beforehand (Hunt & Draper, 1964). Altering the visual cues during meal consumption through the reduction of luminance also alters reported gustatory experiences of the food, such as reported taste and texture (Spence, Okajima, Cheok, Petit, & Michel, 2016; Ueda, Spence, & Okajima, 2020). Hence, visual cues are involved in determining the nutrient content of foods pre-ingestion, modulate the ingestion experience and allow the tracking of nutrient consumption during the meal.

1.2.2.2 – Olfactory Stimuli

Odours are heavily involved in the consumption of food, both before and during ingestion. Before ingestion, volatile compounds that are carried in the air are identified using what is known as orthonasal olfaction, wherein these volatile molecules enter the nasal passage through the nose. As orthonasal olfaction requires olfactory processing before the food is even brought near the oral cavity, it provides information on various properties of the food before ingestion. One such example is the sour smell of spoilt milk caused by the breeding of bacteria. Humans find this odour noxious as the milk is no longer safe to consume, and it is vital to recognise this before any milk has been ingested. As pleasantness of olfactory stimuli does not seem to be modulated strongly by associative learning (Fondberg, Lundström, & Seubert, 2021), this phenomenon appears more innate and may have developed due to strong evolutionary pressure to ensure the safety and adaptive nutrient preferences of food items.

On the other hand, retronasal olfaction occurs upon exhalation. As a result, it typically takes place when foods or drinks with volatile odour compounds are present in the oral cavity, the mastication and swallowing of which would involve exhalation during which time the volatile compounds reach the olfactory epithelium. Retronasal olfaction

has been linked to the sensation of a variety of compounds, including the fat content of oral stimuli (Zhou, Shen, Parker, Kennedy, & Methven, 2016). Crucially, orthonasally tangible odours become intangible when presented retronasally (Rozin, 1982), indicating that there is a duality to the olfactory experience. Retronasal olfaction forms an integral part of the sensation of flavour regarding oral stimuli, whereby this sensation interacts with other food-related sensory modalities, such as taste and texture (Goldberg, Wang, Goldberg, & Aliani, 2018). There is ample evidence of this convergence being highly specific, in that concordant smells, tastes and textures increase the perceived pleasantness of the food item (de Araujo, Rolls, Kringelbach, McGlone, & Phillips, 2003; Fondberg, Lundström, Blöchl, Olsson, & Seubert, 2018; Small et al., 2004; Verhagen, 2007). Pleasantness of combined odour-taste stimuli appears to be modulated by the congruence of the combination of the stimuli, such that a chicken smell followed by a savoury-salty taste was perceived to be more pleasant, as was a sweet-sour taste combined with an orange smell.

1.2.2.3 – Taste

Taste stimuli are inherently involved in the ingestion of food, as the oral cavity is the only area of the body in which taste buds are found. By sensing these taste properties, humans are able to perceive the nutritional content of foods in order to fulfil their nutritional needs. There are five types of taste stimuli widely accepted in the scientific community, namely sweet, sour, salty, bitter and umami (Ikeda, 2002), all of which activate different receptors on the taste buds along the tongue (Trivedi, 2012; Witt, 2019). In general, the receptors affecting the perception of these tastes are distinct, such that it is possible to experience multiple tastes at the same time.

The sour sensation is elicited by acidity in the mouth, as it is known to be present when the oral pH is below 7, meaning that the sour taste receptors respond to the higher concentration of protons in the oral cavity. Sourness is typically found in fruit, such as lemons, oranges and berries, with children exhibiting greater preference for sour food than adults (Liem & Mennella, 2003). On the other hand, saltiness is typically elicited by the presence of certain cations in the oral cavity, specifically monovalent cations such as Na^+ and K^+. Typically, adding sodium chloride (table salt) into food increases the salty taste of the food. Due to the mechanisms of activation of these tastes, it is likely that both saltiness and sourness help regulate the body's supply of ions.

Bitterness is elicited by a variety of compounds, as opposed to a specific ligand or compound. This non-specific sensitivity to even low amounts of bitter substances, in addition to the fact that bitterness is usually found in toxic food items, led to many postulations that this sense evolutionarily developed to avoid toxic food items (Glendinning, 1994). This is further supported by the fact that typically bitter compounds such as quinine often elicit an aversive response in human children and various animal models. However, many adult humans seem to have developed a liking to bitter foods, with bitter ingredients such as coffee and cocoa being popular foods, thereby relaxing the evolutionary pressure on bitter sensitivity (X. Wang, Thomas, & Zhang, 2004).

On the other hand, the sensation of sweetness is evoked when sweet taste receptors, namely the taste 1 receptor family members 2 and 3 (T1R2 and T1R3) are activated by sugars or other compounds, such as artificial sweeteners, some amino acids and some proteins (Chandrashekar, Hoon, Ryba, & Zuker, 2006; A. A. Lee & Owyang, 2017). Typical natural sources of sweetness – that is, sugars – are various ripe fruits, sugar cane and honey, whereas processed foods such as cakes and pastries also tend to be rich in sugar and therefore highly sweet. As the presence of sugar in food is correlated with ripeness, it stands to reason that sugary, and therefore sweet, foods are highly palatable and rewarding, to the extent that infants naturally prefer food stimuli that have a higher lactose content, which is naturally found in breast milk (Desor, Maller, & Turner, 1973; Epstein & Schiffman, 1983).

Traditionally, only sweetness, sourness, saltiness and bitterness were considered primary tastes. However, in the early 1900s, the savoury taste of foods such as cheese and soy sauce was isolated and determined to be a specific taste on its own, known as umami (Ikeda, 2002; McCabe & Rolls, 2007). This savoury taste is elicited when there is a high concentration of glutamate ions in the oral cavity and is highly noticeable when presented with nucleotide-rich foods such as meat, fish, nuts and mushrooms.

The addition of umami to the list of basic tastes also raises the question of whether the currently accepted gustatory stimuli could be expanded with other tastes. One claim that has been introduced in the literature is that humans sense fat content through the triggering of receptors sensitive to fatty acids on the taste buds (Mattes, 2010; Tucker, Mattes, & Running, 2014). However, this ability to taste fat, termed by some authors as oleogustus (Running, Craig, & Mattes, 2015; Running, Mattes, & Tucker, 2013), is widely

disputed (Heinze, Preissl, Fritsche, & Frank, 2015; Keast & Costanzo, 2015). It is known that the fat-sensing mechanism of certain animal models, such as rodents, allow for oral fat sensing to occur due to fatty-acid sensitive chemoreceptors to interact with fatty acids. In the case of primates such as humans and macaques, this is a debatable mechanism because, despite the presence of receptors sensitive to non-esterified fatty acids (NEFAs), the levels of lingual lipase in the primate oral cavity may not be sufficient to allow NEFAs to reach a detectable threshold (Gilbertson, Fontenot, Liu, Zhang, & Monroe, 1997; Gilbertson, Yu, & Shah, 2010; Heinze et al., 2015; B. V. Kulkarni & Mattes, 2014; Running et al., 2013; Tucker et al., 2014), as the main source of fat in the human and primate diet is in the form of triglycerides. In addition, fatty acids are commonly an aversive taste to most humans, such as the example of rancid oil, as opposed to being an appetitive stimulus. In fact, neurons that respond to oral fat content also seem to respond to silicone oil, which does not undergo enzymatic breakdown in lingual lipase into fatty acids (E. T. Rolls, 2011). Therefore, it is more likely that humans sense the fat content of foods, and subsequently assign a reward value to the food, through non-chemical physical properties.

1.2.2.4 – Texture

Texture is inherently a mechanosensory property related to the sense of touch. While texture mostly pertains to one broad sensory modality, it is a highly complex phenomenon, which combines various physical aspects. Most parts of the body are sensitive to touch, including the oral cavity, and it is here we experience the texture of oral food stimuli. The richness of texture parameters within oral food has been explored for decades in the field of food engineering (Bourne, 1975; Friedman, Whitney, & Szczesniak, 1963; Hutchings & Lillford, 1988), with various attempts to characterise important parameters in food texture. Broadly speaking, these characteristics can be grouped into mechanical (pertaining to the forces on various surfaces of the oral cavity), geometrical (pertaining to shape, size and the arrangements of food particles in the oral cavity) and other characteristics (such as the mouthcoating nature or moisture). Notably, while a few characteristics apply more to foods in liquid form compared to those in solid form, dry solid foods still undergo extensive mastication and addition of saliva until a well-lubricated bolus can be swallowed (Chen, 2009).

While the ability for humans to taste fat is disputed (Heinze et al., 2015; B. V. Kulkarni & Mattes, 2014; Mattes, 2010; Running et al., 2013; Tucker et al., 2014), another mechanism by which the fat content of a food is likely to be evaluated is through its texture, as fat has a unique texture different than that of the other macronutrients. In fact, masking the mouthfeel of a fatty stimulus reduces the ability to accurately sense fat (Zhou et al., 2016). Having a non-polar structure while still maintaining a liquid state at body temperature, fat is unable to dissolve normally in saliva during mastication and therefore has unique textural properties that may be the mechanism by which it is sensed. In order to investigate this possibility further, we need to turn to specific fields concerning the study of basic fluid mechanics, such as rheology and tribology.

1.2.2.4.1 – Viscosity

Rheology, the study of how particles flow with respect to each other, has two extremes of behaviour, namely ideal elasticity and ideal viscosity (Koç, Vinyard, Essick, & Foegeding, 2013). Ideal elasticity is characterised by a constant ratio between shear stress and shear strain, that is, the deformation undergone by an area experiencing stress. On the other hand, ideal viscosity is characterised by a constant ratio between shear stress and the strain rate, that is, the degree of deformation per unit time. Most foods exhibit both viscous and elastic properties, although Newtonian liquids such as oils and water tend to display viscous properties more reliably. Owing to this nature, viscosity does provide an inherently reliable textural property by which oral food stimuli, specifically in the liquid or semi-liquid state, can be evaluated.

Unlike amino acids and sugars, dietary fats in the form of triglycerides are non-polar and therefore insoluble in water. The immiscible nature of fat and water allows the fat content of oral food stimuli in liquid or emulsion form to modulate the viscosity of the resultant bolus. Fats are generally more viscous than water, meaning that a great stress needs to be applied in order to achieve the same strain rate as would be needed with water. Therefore, an emulsion with a greater fat content relative to water would be more viscous than one with a lower fat content, as the fat globules add to the overall viscosity of the emulsion. Due to this relationship between fat content and viscosity, the field of food engineering has worked extensively on mimicking the textural properties of fat through the use of non-fat thickeners such as carboxymethylcellulose (CMC) and inulin (Devereux, Jones, McCormack, & Hunter, 2003; Gibis, Schuh, & Weiss, 2015; Zahn, Pepke,

& Rohm, 2010), although modulating viscosity appears to only partially improve the textural properties of non-fat foods. Until only the last few decades, the field of food engineering has very closely examined the rheology of oral food stimuli, with the notion that it is the main textural property of interest pertaining to fat content (Guinard & Mazzucchelli, 1996; Mela, 1988; Shama & Sherman, 1973; Van Vliet, 2002). However, high-viscosity solutions that are not perceived as fat, such as jellies, in addition to the existence of fat-responsive neurons that do not respond to viscosity (E. T. Rolls, Critchley, Browning, Hernadi, & Lenard, 1999; Verhagen, Rolls, & Kadohisa, 2003) imply that viscosity only provides part of the picture and that there are other factors that contribute to oral fat detection in humans. Attempts have been made to attribute this sensation of creaminess to the size of fat globules in the bolus (Guinard & Mazzucchelli, 1996; Kirkmeyer, 2003), although this does not offer a directly measurable parameter and may in fact be more related to sliding friction.

1.2.2.4.2 – Sliding friction

In recent decades, the incomplete link between viscosity and oral fat detection has led to attention being given to involvement of other textural parameters in fat sensing. Specifically, more interest arose in the phenomenon whereby oral fat leaves a lubricating mouth-feel, resulting in a reduction in the oral sliding friction. As opposed to rheology, which measures the flow of a substance with respect to itself, this phenomenon is studied in the field of tribology, which pertains to interacting surfaces in relative motion and examines the role of friction, wear and lubrication. Early attempts to characterise this phenomenon led to the development of the Kokini model, wherein the sensation of oral creaminess was a function defined by both the viscosity and the oral smoothness, which is defined inversely proportional to the sliding friction constant and the load applied (Kokini, 1987; Kokini & Cussler, 1983). However, this work was not explored further until much later, where a sensation of fattiness was described by both a thickness and an absence of roughness (de Wijk, Rasing, & Wilkinson, 2003). Since then, more interest has gone into how the lubricating mouth-feel of fatty foods contribute to fat detection. Chojnicka-Paszun, de Jongh and de Kruif (2012) were among the first to characterise the influence of fat content on the lubricating nature of liquids, where the sliding friction of homogenised milk drinks decreased with increasing fat content above a threshold of 1% fat. This increase was also accompanied by a linear increase in reported creaminess of the liquids, indicating that the tribology of oral liquids is indeed crucial to the sensation

of creaminess. The notion of creaminess is often related to fat content in the subjective description of food items, as the most typical distinction between low- and high-fat foods given by taste panels is that the high-fat foods are described as more creamy (Laguna, Farrell, Bryant, Morina, & Sarkar, 2017). This distinction occurs irrespective of viscosity differences between the low- and high-fat versions of foods (one such example being Greek yoghurt) but is described instead by the tribology of these food items.

The increased attention paid to the role of the tribology in food engineering, especially in the context of fat replacement, has led to many developments in the field. The coefficient of sliding friction, unlike viscosity, is not an inherent property of the fluid but is modulated by the interaction of the fluid and the surfaces involved (Cassin, Heinrich, & Spikes, 2001). Originally focusing on the study of ball bearings and chains, default tribological measurement practices had to be modified to be applicable in biological settings. One such example was the reconceptualization of the canonical friction tests using a rotational motor and a rubber band as surfaces (de Wijk & Prinz, 2005, 2007; de Wijk, Prinz, & Janssen, 2006) or the use of poly dimethyl siloxane (PDMS) as a testing surface. Dresselhuis, de Hoog, Cohen Stuart and van Aken (2008) challenged this notion by comparing the surface properties of smooth glass and PDMS to actual biological tissue in the form of pigs' tongues, concluding that the rough papillae present in tongues as well as the deformability of tissue lead to differences that are not well-replicated by the use of rubber, glass or PDMS. Therefore, in order to truly explore the extent to which tribology influences oral fat sensation, appropriate biological tissue should be used as surfaces. The use of tissue such as pig tongues would allow us to create surfaces that more closely resemble the oral cavity, and this should allow a more accurate measurement of changes to the coefficient of sliding friction (CSF) that is induced by oral food stimuli, especially those containing fat.

1.3 – The Reward Value of Food

Ultimately, when making food-based decisions, the reward value assigned to the options need to be considered, as these choices are performed to maximise the value of foods ingested (Padoa-Schioppa et al., 2006). Given that food is considered a primary reward in itself, there has been a lack of attempt in the literature to specifically identify the components of food, although there have been recent attempts at identifying and characterising the reward components of specific macronutrients, such as fat, in oral food

stimuli (Grabenhorst, Rolls, et al., 2010). More recent studies have looked into the application of the trade-off between reward components through revealed preference theory (Rieskamp, Busemeyer, & Mellers, 2006; Tversky & Simonson, 1993) in the context of bundles of different compositions (Seak, Volkmann, Pastor-Bernier, Grabenhorst, & Schultz, 2021).

One potential meaningful reward principal component of a food item could be nutrient components of the food. As explained in previous sections, nutrients such as fat, protein and carbohydrates are all required to perform basic biological functions, and that the specificity of the drive to eat, in that it is separate from other drives such as to drink when thirsty, may be even more specific, in the sense that one experiences a craving for a certain food because it is rich in the nutrients that are deemed lacking. An example of this can be found in the phenomenon of protein leveraging, where food intake aims to fulfil a quota of protein consumption independent of total caloric consumption, which is observed in animal models (Raubenheimer & Simpson, 2019; Sørensen et al., 2008) and humans (Martinez-Cordero et al., 2012).

Another general trend in macronutrient choice is the highly rewarding nature of fat and carbohydrates, resulting in greater palatability. Rodents appear able to regulate their consumption of chow when fed with diets that are either high in fat or high in carbohydrates, although they gain weight when fed with a mix of these macronutrients (Beilharz, Kaakoush, Maniam, & Morris, 2016; P. M. Johnson & Kenny, 2010). Recent research has indicated that people generally value foods that have a mixture of these macronutrients over those that consist solely of one of them, while controlling for calorie content (DiFeliceantonio et al., 2018). Although the behavioural and neuroimaging components of this study were only conducted on visual stimuli consisting of pictures of the food items used, as opposed to actual oral food stimuli, it still appears that these individual macronutrient components combine to elicit greater subjective values than by themselves. Therefore, it appears that, while primary rewards such as foods can be broken down into smaller components in the form of nutrients, some nutrients display synergistic effects with each other when combined.

The integration of separate reward components in the form of nutrients naturally raises the question of how these nutrients are sensed. Due to nutrient-specific overconsumption arising from signalling-related mutations such as in fat (van der

Klaauw et al., 2016) or in sugar (Søberg et al., 2017), and that these nutrient components are detected through sensory means, it stands to reason that there are nutrient-specific sensory components that contribute to reward value. For example, a rich, creamy milkshake would have sweetness associated with sugar and specific textural properties that are associated with fat such as the viscosity and the sliding friction properties. These specific reward components, however, are largely still unexplored in human neuroscience. These sensory components, especially in the context of how the sensory characteristics of fat contribute to an overall reward value of food, need to be characterised behaviourally and through neuroimaging to identify which brain structures and specific circuits are involved in the process.

1.4 – Neural Systems for Sensory and Reward Processing of Foods

Here, we discuss the neural systems that are involved in both the sensory and the reward processing of food. This section will focus on the structures that are involved in food perception and value processing, discussing the neuroanatomy, functional properties and afferent and efferent connections. Both single-neuron studies in animal models and human functional neuroimaging studies will be discussed, although it is worth bearing in mind that, due to the complexity of delivering oral food stimuli during neuroimaging, most human functional magnetic resonance imaging (fMRI) studies in food perception and reward processing have used conditioned visual stimuli as proxies for actual oral stimuli delivery.

1.4.1 – Insula

1.4.1.1 – Anatomy and connections

One of the main areas that are likely to be involved in oral stimulus processing is the insula, first described by Reil (1809) as the island-like region within the lateral fissure. Mesulam and Mufson conducted one of the most comprehensive architectonic investigations into the insula, describing its connections and cytoarchitecture using the brains of both rhesus macaque monkeys and a human (Mesulam & Mufson, 1982a, 1982b; Mufson & Mesulam, 1982). Notably, the human insula is highly similar in terms of general architectonic plan to that of the macaques, albeit with more details such as two distinct gyri and several sulci (Mesulam & Mufson, 1982a), thereby implying that functional properties observed in single-cell macaque studies may also bear some significance in humans.

The limen insulae is the junction point of the anterior and posterior stem of the lateral sulcus, dividing the insula into what is known as the anterior insula and the posterior insula. One noteworthy observation is that the architectonic properties of the insular cortex on either side of the limen insulae are highly distinct, with an increase in granularity as one moves posterior from the agranular anterior insula (Mesulam & Mufson, 1982a). The anterior insula is composed of an inner and an outer stratum, where the neurons of the inner stratum connect to the claustrum and the outer stratum neurons project to the pyramidal neurons of the prepiriform cortex. Notably, while the anterior insula begins along with the superior limiting sulcus along the frontoparietal operculum, there is no well-defined boundary between the ventral insula and the OFC, with the exception of the existence of a small orbito-insular sulcus in some brains.

Sweetness, along with the other basic tastes of saltiness, bitterness, sourness and umami, is transmitted from the taste receptors through gustatory fibres toward the pons, nucleus of the solitary tract, and the parvocellular part of the thalamic ventral-posterior medial nucleus (VPMpc), and terminating in the anterior insula/frontal operculum (Ito & Ogawa, 2005; Ogawa, 1994; Ogawa, Ito, & Nomura, 1985; Pritchard, Hamilton, Morse, & Norgren, 1986), although humans and non-human primates do not appear to have the pontine taste area present in rodents. The direct projection from the thalamic taste nucleus VPMpc leads to the anterior insula and frontal operculum to be designated as the primary taste cortex (Ogawa, 1994; Scott, Yaxley, Sienkiewicz, & Rolls, 1986), which is responsive to stimulation of the taste nerve instead of the lingual nerve.

On the other hand, the posterior insula has much more demarcated layers similar to other isocortical areas. In addition to more granular cortex, especially in the most caudal and dorsal regions, the part of the insula posterior to the limen insulae is also characterised by more tangential fibres in the infragranular region and substantially more intracortical myelin than the agranular insula (Mesulam & Mufson, 1982a). The posterior (granular) insula is bounded ventrally by the medial limb of the inferior limiting sulcus. The area between the anterior and the posterior insula, also known as the mid-insula, displays a blend of these properties, with dysgranularity and some stratification. Due to these vast differences in cytoarchitecture, it is likely that the granular, dysgranular and agranular segments of the insula are involved in the processing of different types information.

Tractographical studies have also shown vastly different connections pertaining to the anterior and posterior insula. The agranular anterior insula is the only area that receives inputs from both the prepiriform olfactory cortex and the frontal operculum, meanwhile the granular portion of the posterior insula receives many inputs from the anterior inferior parietal cortex and somatosensory areas in SI and SII (Mufson & Mesulam, 1982). One ought to also note that both areas receive inputs from the OFC, although overall the agranular insula receives more input from the frontal lobe. While these inputs are largely reciprocal, both anterior and posterior parts of the insula also project to areas of the limbic system such as the amygdala (Mesulam & Mufson, 1982b). Therefore, one is able to see that olfactory and gustatory connections are limited to the anterior insula, whereas the posterior insula appears to be more connected with auditory and somesthetic structures.

1.4.1.2 – Functional properties of single neurons

From single-neuron stimulation and ablation studies in macaques, we know that the insula is involved heavily in the processing of gustatory stimuli, with the presence of taste-sensitive neurons in the structure (Bagshaw & Pribram, 1953; Yaxley, Rolls, & Sienkiewicz, 1990). In one of the most important studies on the function of the insular cortex in the encoding of oral stimuli, Verhagen, Kadohisa and Rolls (2004) found that insular and opercular neurons encode other properties of oral stimuli in addition to taste, such as viscosity, temperature and fat content. Out of the 68 orally responsive neurons recorded, almost half (31) of these neurons respond to a single modality (taste, viscosity, temperature or fat), with substantially more neurons encoding taste (15) or viscosity (12) than the other two modalities. Notably, almost 30% of these neurons encoded two modalities, with there being an equal number of neurons (6) responsive to both taste and temperature, both taste and viscosity and both temperature and viscosity, whereas only one neuron was found to encode both fat content and viscosity. There were also 6 neurons responsive to taste, temperature and viscosity, whereas two neurons were responsive to all 4 modalities. These findings imply that the characteristics of oral stimuli, such as their temperature, fat content, taste and viscosity, are represented largely unimodally in insular and opercular neurons, with combinations of these modalities also being represented to a smaller extent. Indeed, using multidimensional scaling and dendrogram analysis in the above study, the first branch of dissimilarity was noted to be between low-viscosity stimuli (such as blackcurrant juice and a quinine solution) and

high-viscosity stimuli (oils and a CMC series). The second branch then splits the CMC series from the oils. Of note, fatty acids such as lauric acid and linoleic acids were clustered with the low-viscosity gustatory stimuli, indicating that responses to fat were due to texture and not the breakdown of triglycerides into fatty acids by lingual lipase. Interestingly, while more taste-encoding neurons were found in the anterior portions of the insula, more neurons encoding the somesthetic characteristics of the oral stimuli were found as one progressed posteriorly.

In contrast, neurons in the granular posterior insula appear to encode innocuous somatosensory stimuli (C. J. Robinson & Burton, 1980a), to the extent that over a third of the neurons sampled in this region responded robustly to very light touch or low-frequency vibrations (C. J. Robinson & Burton, 1980b). Furthermore, neurons that encoded fat content independently of viscosity in the granular insula also appeared to respond to the CSF (E. T. Rolls, Mills, Norton, Lazidis, & Norton, 2018). This study re-examined the neural responses characterised in Verhagen, Kadohisa and Rolls (2004), characterising fat-responsive neurons into neurons linearly correlated with decreasing CSF, neurons non-linearly correlated with decreasing CSF, neurons correlated with increasing CSF and neurons responsive to viscosity irrespective of fat content. In the agranular insular primary taste cortex, only a small proportion of neurons corresponded to decreasing CSF (less than 10%), whereas about 10% responded to viscosity regardless of fat and a large number (about 25%) responded to increasing CSF – that is, they were inhibited by the presence of oral fat – indicating that the agranular insula does indeed focus more on taste-related stimuli than on textural properties, especially in the case of sliding friction. Population decoding analysis techniques trained on CSF were able to predict CSF with 100% accuracy with 8 neurons, whereas 11 neurons trained on viscosity were unable to predict oral CSF content beyond chance level, indicating that these textural parameters occur through distinct channels. Taken together, these findings indicate that pure gustatory and pure somesthetic information are processed separately and in parallel in these two regions of the insula.

1.4.1.3 – Evidence from functional neuroimaging

Although lesion studies involving gustation are sparse, neuroimaging studies have indicated BOLD activation in similar areas (Small, 2006; Small et al., 1999), which implies that the human gustatory pathway is similar to those of non-human primates. In order to

investigate encoding of oral stimuli in humans, de Araujo and Rolls (2004) presented a range of oral stimuli to humans in an fMRI setting, trying to isolate areas of the brain that represent textural and taste stimuli. In doing so, they highlighted that the anterior insula responds to both sweet-taste and viscosity, as formally tested using conjunction analysis. Furthermore, fat and other viscous stimuli also elicit responses in the mid-insula, posterior to the primary taste cortex, independent of sugar activation, indicating a specific fat/viscosity-processing area separate from that of taste. This further suggests that fat-sensing occurs in parallel, but separate to, taste processing through the perception of the viscosity of the oral stimulus in the context of humans.

There is also ample evidence for the role of the insula, especially the anterior agranular insula, in the reward processing of food-related visual cues (de Araujo, Geha, & Small, 2012). Specifically, activations in the anterior insula correlate with postingestive hedonic conditioning (de Araujo et al., 2013). Furthermore, the anterior insula is also implicated in highly specific cravings, where the identity of the craved food substance is pivotal (Alonso-Alonso et al., 2015; Pelchat, Johnson, Chan, Valdez, & Ragland, 2004). Differences in activations in response to food-related visual cues also seem to be modulated by individual body mass index (BMI), where obese individuals exhibit greater response to visual representations of high-calorie food compared to lean controls (Rothemund et al., 2007; Stoeckel et al., 2008).

On the other hand, functional neuroimaging evidence indicates that the posterior insula is associated with non-taste-related stimulation, such as gastric distention without ingestion (G. J. Wang et al., 2008). Moreover, there is greater connectivity between the OFC and the posterior insula during processing of non-food stimuli in a state of hunger (Charroud et al., 2021). Furthermore, the posterior insula also displays greater functional connectivity with somatosensory areas (Cauda et al., 2011). However, there is also evidence of the granular posterior insula being recruited in the processing of the taste pleasantness of oral stimuli, whereas the anterior insula was more strongly correlated with the presence of a taste stimulus (Dalenberg, Hoogeveen, Renken, Langers, & ter Horst, 2015).

1.4.1.4 – Proposed function and open questions

Taken together, these findings highlight that the insular cortex should be viewed as at least two functional structures with related but partly distinct information processing,

where tastes are more pertinent to the anterior insula and textural components more pertinent to the posterior insula (Fig. 1.1), which suggests that taste and somatosensory information is relayed through separate or partly separate channels. In single-cell studies, we see that neurons responsive to fat independent of viscosity appear to also be responsive to the coefficient of sliding friction (E. T. Rolls et al., 2018). However, insular encoding of this critical textural component has yet to be demonstrated in humans. Therefore, in this thesis, we expect to see that both viscosity and coefficient of sliding friction are processed in the insula, mostly the posterior insula, whereas taste stimuli would be processed in the anterior portion of the insula. This also raises the question of where the information is transmitted in order to generate a reward value, as the insula also projects to reward systems such as the amygdala and the OFC.

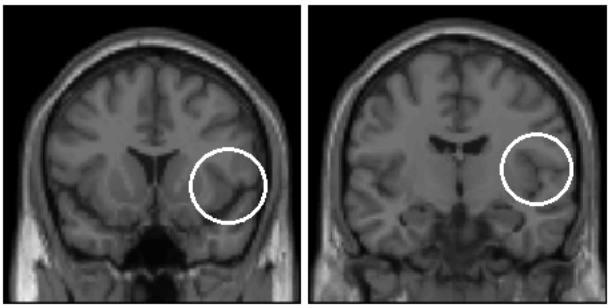

Figure 1.1. *Left* Region of interest for anterior insular taste cortex (y = 10). *Right* Region of interest for posterior insula (y = -8)

1.4.2 – Orbitofrontal Cortex

1.4.2.1 – Anatomy and connections

The orbitofrontal cortex (OFC) is located within the prefrontal cortex, just behind the orbits. Initial investigations done on macaque brains indicate that the primate OFC houses many small areas that are highly varied in both structure and connections (Carmichael & Price, 1994; Öngür & Price, 2000), to the extent that the bound between the agranular insula and the OFC is often blurred (Preuss & Goldman-Rakic, 1991). Later tractographical studies into the human brain have shown that there are many shared similarities between the non-human primate OFC and that of humans (Öngür, Ferry, & Price, 2003), allowing us to use single-neuron recordings in non-human primates such as macaques to inform us of putative networks and functions within the human brain.

Brodmann (1914) only identified areas 11 and 47 within his work, with the lack of a definite border between the OFC and surrounding areas such as the agranular insula

(Öngür et al., 2003). Much more recently, areas 13, 14 and 12 were categorised to be in the OFC (Carmichael & Price, 1994; Mesulam & Mufson, 1982a; Öngür et al., 2003), and 3-D parcellation techniques allowed more precise subdivision of area 47 into two medial and two lateral sub-areas, in addition to an cytoarchitectural gradient in the anterior-posterior direction (Uylings et al., 2010). This gradient manifests through a reduction in granule cells within layer IV of the OFC, causing the posterior segments of areas 13, 14 and 47/12 to be dysgranular or agranular. Due to the lack of a definite boundary, the medial prefrontal cortex and the OFC are often treated as one unity, until it was later established that two distinct networks exist and are spatially defined within the regions (Öngür & Price, 2000). Therefore, the OFC should be examined in its own right.

The OFC receives afferent projections from all sensory modalities, including visual, olfactory, somatosensory and taste (E. T. Rolls & Baylis, 1994; E. T. Rolls & Grabenhorst, 2008). A large number of direct projections from the primary taste cortex into a lateral part of the OFC indicates that the OFC may be considered a secondary taste cortex (Baylis, Rolls, & Baylis, 1995). Olfactory neurons are more likely to be found in the mid-orbitofrontal cortex area, however, where there are direct inputs from the piriform cortex to the posterior orbitofrontal cortex, which then projects onwards to the mid-orbitofrontal cortex (Barbas, 1993; Morecraft, Geula, & Mesulam, 1992). Visual inputs reach the OFC through the inferior temporal cortex and the temporal pole (Barbas, 1988; Barbas & Pandya, 1989; E. T. Rolls, 2007). Meanwhile, the OFC also notably receives inputs from somatosensory cortical areas such as the insula, the frontal and pericentral operculum and SII (Barbas, 1988; Carmichael & Price, 1994). The convergence of so many sensory inputs into the OFC may indicate that sensory information is integrated and evaluated here. This is further supported by its reciprocal connections to other structures involved in eating, reward and learning such as the amygdala (Barbas, 2007), ventral striatum (Ferry, Öngür, An, & Price, 2000) and lateral hypothalamus (T. N. Johnson, Rosvold, & Mishkin, 1968).

1.4.2.2 – Functional properties of single neurons

Single-neuron recordings in the OFC of behaving macaques have implicated the region in the processing of rewards, as can be expected from its inputs from various sensory modalities and reciprocal connections with limbic systems. Such examples include responsiveness of visual (E. T. Rolls, Critchley, Mason, & Wakeman, 1996) and olfactory

(Critchley & Rolls, 1996a; Tanabe, Iino, Ooshima, & Takagi, 1974) stimulation. Within functions specifically pertinent to this thesis, OFC neurons are known to respond to gustatory stimulation, with a range of locations from the lateral OFC (Yaxley et al., 1990) to the medial OFC (Critchley & Rolls, 1996b; Kadohisa, Rolls, & Verhagen, 2005; Pritchard et al., 2005). Crucially, neurons in the OFC have also been shown to respond to the textural properties of food, such as its viscosity, grittiness and heat as sensed by capsaicin (Kadohisa, Rolls, et al., 2005; E. T. Rolls, Verhagen, & Kadohisa, 2003). Initial findings highlighted several OFC neurons that encoded the fat content of a stimulus regardless of its viscosity (Verhagen et al., 2003), although much more recently these neurons have been confirmed to encode the sliding friction of stimuli (Fig 1.2; Rolls et al., 2018) as examined through single-cell responses of primates who are given a CMC viscosity series as well as non-fat oils, namely mineral oil and silicone oil in addition to fatty stimuli. Interestingly, these fat-responsive neurons did not exhibit responses to free fatty acids, such as lauric acid and linoleic acid, further reinforcing their tuning towards the texture of high-fat oral stimuli as opposed to the receptors sensitive to fatty acids. Notably, OFC neurons also display less correlated responses to taste stimuli in the OFC than in the insula or the amygdala, which indicates a much finer tuning of each neuron to particular stimuli. Furthermore, dendrograms of the neural responses clustered stimuli firstly based on sugar content (singling out glucose and blackcurrant juice), secondly on sodium content (singling out sodium chloride and monosodium glutamate) and only thirdly based on viscosity, before subsequently differentiating fat-containing stimuli from a high-viscosity CMC series (Kadohisa, Rolls, et al., 2005). In addition, the OFC has a more equal distribution of unimodal, bimodal and multimodal neurons (30%, 30% and 28% respectively) in contrast to areas such as the amygdala and the insular taste cortex where unimodal neurons are substantially more prevalent. This broad tuning of stimulus responses in the OFC implies that the OFC is likely to be the integrator hub of sensory information on the taste and texture of oral food stimuli, where each particular oral stimulus has a much more distinct representation in the OFC than it would in areas such as the insula and amygdala.

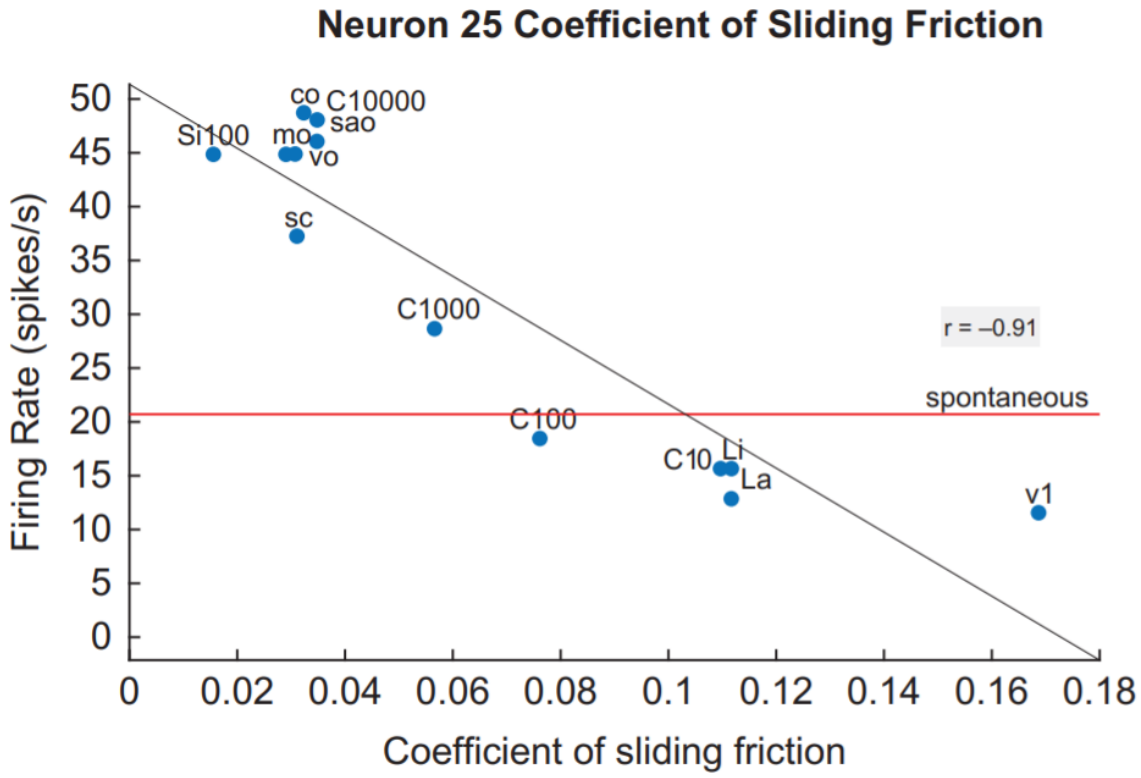

Figure 1.2. Orbitofrontal neuron negatively coding sliding friction. Abbreviations: Si100 – silicone oil; co – coconut oil; mo – mineral oil; C10000 – CMC (10000 cP); sao – safflower oil; vo – vegetable oil; sc – single cream; C1000 – CMC (1000cP); C100 – CMC (100cP); C10 – CMC (10cP); Li – linoleic acid; La – lauric acid; v1 – water. Reproduced from Rolls et al. (2018)

OFC neurons are also known to encode the economic value of objects. Using a binary choice task, Padoa-Schioppa and Assad (2006) showed that neurons in the OFC encode the economic value of offered and chosen goods. Using choices between varying amounts of water and juice, they expressed the value of one good (juice) relative to the other (water) and discovered OFC neurons that encoded the value of the offered goods in addition to neurons that encoded the value of the chosen good, with more neurons encoding the offered values than the chosen value, which implies that there is a transition in the OFC from encoding the value of offered objects to the decision between the two objects. Interestingly, adding a third stimulus such that there are three distinct stimulus pairs does not change the encoding of the original pairing (Padoa-Schioppa & Assad, 2008). This menu-invariant transitivity suggests that OFC value signals are held consistent over time. In addition, Lesions in the OFC of macaques are known to impair reward learning and updating (Iversen & Mishkin, 1970; Izquierdo & Murray, 2004). Food preferences, as well as emotional executive control, are heavily affected by lesions

in orbital and frontal areas (Butter, Mc Donald, & Snyder, 1969; Butter, Snyder, & McDonald, 1970). Such changes, as well as euphoria and impulsivity, are documented in humans with orbitofrontal damage (Berlin, 2004; Berlin, Rolls, & Iversen, 2005), possibly stemming from an impairment to update the assignation of reinforcing stimuli (Bechara, 2000; Hornak et al., 2004). Hence, evidence indicates that the OFC is integral in reward processing, especially in the case of food intake, as OFC neurons encode reward value of the food item during ingestion in addition to the food item to be chosen.

1.4.2.3 – Evidence from functional neuroimaging

Similar to the findings in single-cell recordings, human fMRI studies have also implicated the OFC in various reward processes. Specifically within the context of food-based rewards, real-time oral food rewards have been documented to elicit activations in the OFC, as they are primary rewards (Grabenhorst, Rolls, et al., 2010; Small et al., 1999). Such activations seem to be elicited by both the taste (Francis et al., 1999; Frey & Petrides, 1999; O'Doherty, Rolls, Francis, Bowtell, & McGlone, 2001) and the textural (de Araujo & Rolls, 2004; Grabenhorst & Rolls, 2014) aspects of oral food stimuli. OFC activations were recorded in response to oral fat (in the form of vegetable oil) and high-viscosity stimuli (through a CMC viscosity series), as examined through a conjunction analysis (de Araujo & Rolls, 2004). In order to tease apart the responses arising purely from fat content and those from reward value, Grabenhorst et al. (2010) used a 2×2 factorial design with high- and low-fat stimuli of either pleasant (vanilla) or unpleasant (strawberry) flavours. In doing so, they discovered that activations in the lateral OFC were correlated with the unpleasantness of fat texture, that is, the lateral OFC exhibited greater BOLD activation in response to fat content and was negatively correlated with reported pleasantness, whereas mid OFC activations were correlated with the pleasantness ratings when contrasting high- and low-fat oral stimuli. This study suggests that, similar to the single-neuron studies into oral food texture (Kadohisa, Rolls, et al., 2005) and reward (Padoa-Schioppa & Assad, 2006), there is a gradation in OFC activity in the encoding of fat texture without representing its value (in the lateral OFC) and a representation of fat and the reward value of the food stimulus (in the medial OFC). Further, OFC activations also seem to encode the reward value of foods even when presented as visual stimuli. A Becker-DeGroot-Marschak (BDM) auction task (Becker et al., 1964) demonstrated that the caloric density of visually presented food items were correlated with their subjective value, which in turn was correlated with BOLD activation in the OFC during the visual

presentation of these foods (DiFeliceantonio et al., 2018; Tang, Fellows, & Dagher, 2014). In this task, participants are asked to place a bid for a reward that is then compared to a randomly generated computer bid, where winning the bid leads to the participant paying the amount they bid in exchange for the reward, which reflects the true value that they assign to the reward as it avoids both overspending and underspending. These findings, in addition to the representation of the convergence of taste and olfactory stimuli into flavour in the OFC (de Araujo et al., 2003; Seubert, Ohla, Yokomukai, Kellermann, & Lundström, 2015), indicate that the OFC integrates various sensory modalities to process the overall value of rewards.

One advantage of human fMRI studies is the ability to image several structures almost simultaneously, albeit at the cost of spatial and temporal resolution. This allows functional connectivity analyses, which are able to demonstrate how activations within certain parts of the brain correspond to those of others. Grabenhorst and Rolls (2014) demonstrated using psychophysiological interaction (PPI) analysis that functional coupling between the OFC and the oral somatosensory cortex (SSC) increased upon tasting pleasant fatty stimuli. Moreover, the OFC also exhibits greater functional coupling with the insular taste cortex when evaluating oral food stimuli based on their pleasantness as opposed to their intensity (Luo, Ge, Grabenhorst, Feng, & Rolls, 2013), indicating that the OFC is indeed involved in hedonic processing of reward stimuli from different modalities.

Notably, the OFC is known to encode the identity of reward stimuli. Multivariate pattern analysis (MVPA) techniques have been able to show that there is identity-specific encoding of olfactory rewards in the OFC, as a support vector machine (SVM) decoder was able to differentiate between sweet and savoury olfactory rewards from distributed OFC BOLD activation patterns (Howard, Gottfried, Tobler, & Kahnt, 2015). In this study, a 2×2 factorial design between sweet/savoury odours and high/low values were used, which was able to then establish that the value signals in the OFC were specific to either the sweet or the savoury odours. This decoding accuracy is in contrast to that of the ventromedial prefrontal cortex (vmPFC), where the decoding accuracy was independent of the identity of the odour. Functional connectivity analyses indicated that the identity-specific and identity-general signals had functional connections to distinct structures, with identity-general signals in the vmPFC being coupled with the amygdala and the

identity-specific signals in the OFC showing coupling with the piriform cortex, thereby reinforcing the notion that the OFC receives inputs from primary sensory areas (in this case, the piriform cortex) to compute an identity-specific reward value.

The gradation of representation of rewards in the OFC has also been noted using MVPA techniques. Suzuki, Cross and O'Doherty (2017) presented participants with pictures of food stimuli of varying macronutrient compositions and performed a BDM auction with the payout of the food item won in the auction after the scanning session. In doing so, they reported that both the lateral OFC and the medial OFC encoded subjective value of the food items presented. However, an SVM decoder was also able to decode the individual macronutrient content of the food items in the lateral OFC, which was not possible from the patterns of the medial OFC, indicating a gradation of reward representation where the lateral OFC encodes the various reward components, in this case macronutrients, of food reward stimuli whereas the medial OFC has a less distinct representation of the macronutrient space of the food items. This encoding in the lateral OFC was noted for both objective nutrient content and subjective perceptions of nutrient content (what participants thought each food item contained), which was also notably missing in the medial OFC. These results, taken together with previous knowledge of OFC encoding of specific nutrients such as fat (Grabenhorst, Rolls, et al., 2010) and the identity-specific encoding of rewards (Howard et al., 2015), suggest that the OFC does indeed integrate the different reward component of foods into an identity-specific reward value. However, this particular study relied on the previous knowledge of participants of well-known store-brand food items and the use of visual stimuli. Therefore, the question of how previously unknown reward identities are integrated in the OFC, as well as the mechanisms behind the nutrient detection of foods already in the oral cavity and how they contribute to reward signals in the OFC, is still outstanding.

1.4.2.4 – Proposed function and open questions

The afferent projections into the OFC, the responsiveness of OFC neurons to various sensory modalities and the representation of subjective reward value in the OFC indicate that the OFC integrates reward values from various sources. The independence of the encoding of reward from the other rewards on offer (Padoa-Schioppa & Assad, 2008) implies that this assignation is done to an absolute common currency against which to compare rewards, as opposed to encoding subjective value relative to what is present.

Kadohisa et al. (2005) report that there are fewer correlations between taste stimuli in neurons within the OFC compared to neurons in the insular taste cortex, implying that there may be more discrete encoding in the OFC that relies on population-based patterns. If the OFC were indeed a higher-level processing area of reward value from basic stimuli, more fine-tuned representations of rewards would be expected. Furthermore, both the identity-specific value encoding (Howard et al., 2015) and component-based encoding of nutrient content (Suzuki et al., 2017) in OFC neurons are also population-based. Therefore, it is likely that, in response to specific nutrients in the oral cavity such as fat or sugar, OFC responses would be population-based. However, the broad tuning of OFC neurons in response to specific orally delivered stimuli have yet to be fully explored.

The role of the OFC as a location for the representation of flavour due to its ability to converge taste and olfactory stimuli into a coherent flavour representation (de Araujo et al., 2003; Seubert et al., 2015) does raise the question if this also applies for somatosensory stimuli. As the lateral OFC also appears to encode some textural properties such as viscosity in humans (de Araujo & Rolls, 2004; Grabenhorst & Rolls, 2014; Grabenhorst, Rolls, et al., 2010) and the coefficient of sliding friction in macaques (E. T. Rolls et al., 2018), it stands to reason that the lateral OFC also encodes the coefficient of sliding friction in humans. Furthermore, it would also integrate these somatosensory signals along with taste and olfactory stimuli into a coherent flavour signal, which would then be processed into subjective reward value in the medial OFC (Kringelbach, 2004; Padoa-Schioppa & Assad, 2006; Suzuki et al., 2017).

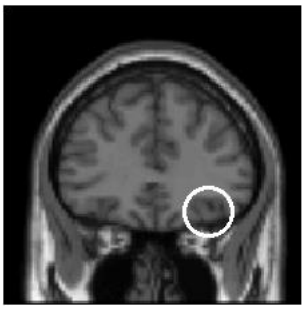

Figure 1.3. Region of interest for orbitofrontal cortex

1.4.3 – Oral Somatosensory Cortex

1.4.3.1 – Anatomy and connections

The somatosensory cortex (SSC) extends across the post-central gyrus and parts of the parietal operculum. Areas 1, 2 and 3b are collectively known as the primary somatosensory cortex (SI), posterior to which lie the parietal ventral area and the second somatosensory area (SII). The organisation is such that the SSC on each hemisphere corresponds to the contralateral half of the body, and that the upper part corresponds to the lower limbs and the head and face are mapped onto the lower section. Specifically, the lower section of the SSC, mapping the oral cavity, will be discussed within the scope of this thesis, which encompasses the Brodmann areas 1, 2, 3a and 3b (Kaas, 1983; Merzenich, Kaas, Sur, & Lin, 1978).

Sensory fibres from the oral cavity reach their cell bodies in the trigeminal ganglion at the bottom of the middle cranial fossa, from which they then project to the trigeminal nuclei in the brainstem on the pontine level and subsequently the thalamus and the cortex (Haggard & de Boer, 2014; Walker, 1990). Notably, the tactile inputs from the oral cavity project into the oral SSC from the most medial division of the ventral posterior nucleus (VPM), whereas taste inputs on the tongue are relayed through the parvocellular VPM nucleus (VPMpc) into the primary gustatory area (area G), indicating that the thalamocortical projection to the tongue area of 3b is tactile in nature as opposed to being chemosensory (Cerkevich, Qi, & Kaas, 2014; Kaas, Qi, & Iyengar, 2006). Furthermore, there appears to be substantial interconnectivity between different orofacial representations in area 3b, in addition to reciprocal connections to SII, the parietal ventral, parietal rostral and ventral somatosensory areas (Cerkevich et al., 2014).

1.4.3.2 – Functional properties of single neurons

Single-neuron electrophysiological studies have indicated that a relatively large portion of area 3b responds to somatosensory stimulation of the face and the oral cavity in non-human primates such as squirrel monkeys and macaques (Manger, Woods, & Jones, 1995, 1996). Basing their work on these data, Cerkevich, Qi and Kaas (2013) used a combination of electrophysiology, electrical stimulation and histology to establish that area 3b has a richly detailed representation of the oral cavity from mechanical stimulation (Fig. 1.4). Each section of the oral cavity had its respective somatotopic representation in area 3b. However, receptive fields are much larger in area 2, just caudal of area 3 (Toda & Taoka, 2001), implying a rostrocaudal convergence of somatosensory neurons (Toda & Taoka, 2002, 2004). This convergence appears to integrate information from a variety of oral tissues, such as lingual, labial and gingival tissue, which could be due to simultaneous stimulation during typical oral activities such as mastication or ingestion (Toda & Hayashi, 2010). However, despite the evidence of oral SSC encoding of touch in the oral cavity, oral SSC responses to specific food properties, such as its viscosity or sliding friction, have yet to be explored.

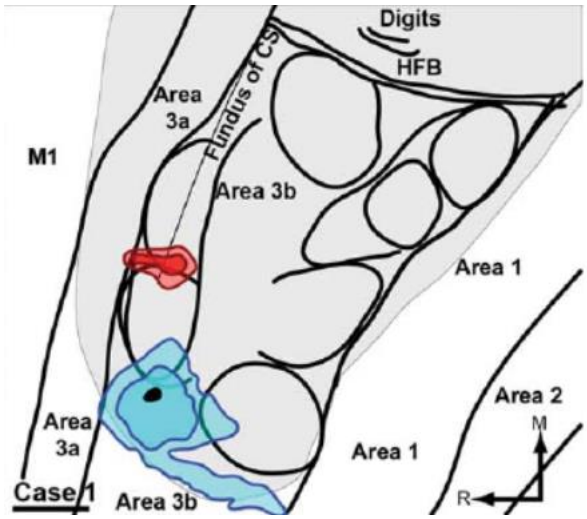

Figure 1.4. Rich representation of orofacial somatosensory system in area 3b. Red region corresponds to touch and injection behind upper teeth area, blue corresponds to touch and injection on the tip of the tongue. Figure reproduced from Cerkevich et al. (2013).

1.4.3.3 – Evidence from functional neuroimaging

The mapping of the oral SSC has been explored using various imaging techniques, such as electroencephalography, magnetoencephalography and fMRI (Karhu, Hari, Lu, Paetau, & Rif, 1991; Minato et al., 2009; Miyamoto et al., 2006; Nakamura et al., 1998; Sakai et al., 1995; Tamura, Shibukawa, Shintani, Kaneko, & Ichinohe, 2008), showing that oral cavity

stimulation corresponds to activations in the SSC more inferior and lateral than any other body part. Similar to the single-neuron recordings, fMRI has also shown that the mapping in the oral SSC is highly detailed and localised, with anterior and posterior lingual stimulation eliciting different activations (Sakamoto, Nakata, Inui, et al., 2010; Sakamoto, Nakata, Yumoto, & Kakigi, 2010). Further, the oral SSC is activated by tactile stimuli independent of taste, indicating that taste stimuli is not processed in the oral SSC (Miyamoto et al., 2006). Specifically within the context of food ingestion, the oral SSC has been shown to be activated upon consumption of liquid food rewards (Eldeghaidy et al., 2011; Stice, Burger, & Yokum, 2013), where the activation increases after repeated exposure to liquid food rewards (Sadler et al., 2021), suggesting that this response arises due to the presence and processing of liquid food items in the oral cavity.

Part of the OFC has been reported to correspond to pleasant oral fat delivery, specifically when participants are given oral stimuli consisting of where the activity is correlated with greater pleasantness ratings of the fat texture (Grabenhorst, Rolls, et al., 2010). In order to further explore the role of the oral SSC in the specific processing of oral food delivery, and the putative mechanism by which oral fat sensation occurs, Grabenhorst and Rolls (2014) conducted PPI analysis that determined greater functional coupling between the oral SSC and the OFC during the processing of pleasant fatty stimuli. However, this coupling was not observed when fat content was increased but with the addition of the less pleasant strawberry flavour, nor was it present when comparing the two low-fat stimuli of different flavours. This indicated that the coupling did not depend unilaterally on the increase in fat content or on merely changing to a more pleasant flavour, instead requiring the combination of two components, the fat content and a consonant flavour, for the increased reward component to be detectable in the PPI analysis. This may indicate that the oral SSC processes the somesthetic mouthfeel of the fatty stimulus, upon which this information is relayed to the OFC for reward integration and processing.

1.4.3.4 – Proposed function and open questions

From the overall body of evidence, the oral SSC appears to encode somesthetic information within the oral cavity, with a richly detailed mapping. Within the context of this thesis, it is likely that textural properties of fat, namely the viscosity and the sliding friction of fatty oral stimuli, would be processed in somesthetic areas such as the oral SSC,

before this information is forwarded to the OFC which integrates the various sensory inputs into a universal reward value for the stimulus Interestingly, while there have been a few studies into the oral SSC and adjacent areas in processing of fat and viscosity (de Araujo & Rolls, 2004; Grabenhorst & Rolls, 2014), the question of why viscosity-independent areas still respond to oral fat stimuli still stands. One potential mechanism by which this viscosity-independent activity may occur is that the oral SSC and granular insula, being somesthetic processing areas, also encode the coefficient of sliding friction of the oral stimulus, which would then allow areas such as the OFC to compute its fat content and assign an appropriate reward value.

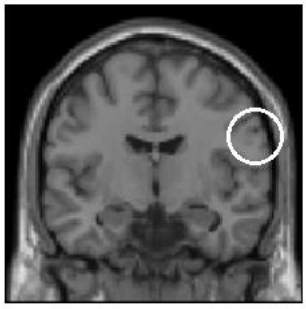

Figure 1.5. Region of interest for oral somatosensory cortex.

1.4.4 – Lateral Hypothalamus

1.4.4.1 – Anatomy and connections

The hypothalamus is part of the diencephalon, along with the epithalamus, dorsal thalamus and the ventral thalamus (Herrick, 1908, 1910), where it occupies the most ventral section thereof. Caudal to the hypothalamus lies the periventricular and tegmental grey matter, which is part of the mesencephalon. Typically, the hypothalamus is divided into the periventricular, medial and lateral hypothalamus, mediolaterally arranged. The hypothalamus is composed of several prominent and interconnected nuclei, within which neurosecretory elements are found to regulate hormonal systems within the body.

Due to the many connections between the hypothalamus and other brain structures, we will mention only those pertinent to the scope of this thesis, that is, the areas involved in food intake regulation and reward processing. Beyond connections between its own nuclei, the hypothalamus also has a wide network of connections with other areas of the brain, especially other structures within the limbic system such as the amygdala and the hippocampus. This connection emerging from the hypothalamus takes

the form of the medial forebrain bundle, one of the components of which consists of dopaminergic neurons projecting from the ventral tegmental area to the nucleus accumbens (You, Chen, & Wise, 2001). There are also several reciprocal connections to the neocortex, such as to the orbitofrontal cortex and the cingulate cortex.

1.4.4.2 – Functional properties of single neurons

The hypothalamus is a key brain region involved in the regulation several fundamental biological functions, such as hormonal regulation (R. Hall & Gomez-Pan, 1976), thermoregulation (Van Tienhoven, Scott, & Hillman, 1979; Zhao et al., 2017), osmoregulation – through thirst and diuretics (A. K. Johnson & Thunhorst, 1997; Verney, 1947) – and feeding regulation (Morton, Cummings, Baskin, Barsh, & Schwartz, 2006; Zhan et al., 2013; Zhang et al., 1994). Due to these many roles that it plays, this thesis will only focus on the role of the hypothalamus in the regulation of appetite and feeding.

One of the earliest appetite-related hormones discovered was leptin, which has receptors expressed mostly within the hypothalamus (Zhang et al., 1994). Leptin-induced overfeeding can occur through either the insufficient leptin production or a developed resistance to leptin as a signal. This pattern can be observed in the single-cell extracellular recordings in the macaque lateral hypothalamus, which responds to operant feeding behaviour and is modulated by the palatability of the food and satiation (Fukuda, Ono, Nishino, & Nakamura, 1986). Notably, the lateral hypothalamus responses seem to regulate food intake and is affected by satiety (E. T. Rolls, Murzi, Yaxley, Thorpe, & Simpson, 1986). Interestingly, due to the reciprocal connections between the amygdala and the lateral hypothalamus, interrupting this pathway by suppressing amygdala neurons appears to reduce the firing rate of a few lateral hypothalamus neurons, mostly those that respond to the sight of food, which implies that the lateral hypothalamus is mostly involved in feeding regulation and not directly involved in learning (Fukuda & Ono, 1993).

1.4.4.3 – Evidence from functional neuroimaging

Functional neuroimaging of the role of the hypothalamus in feeding behaviour has shown that activity of the hypothalamus indicates craving for food and that this craving-related activity can be attenuated by feeding, to the extent that this attenuation is much more effective when the nutrient is ingested orally instead of injected into the bloodstream (Smeets et al., 2007). Individuals who regularly consume a greater amount of calories,

such as obese individuals, tend to display a reduced attenuation of hypothalamic activity upon oral ingestion (Matsuda et al., 1999). Interestingly, activity in the hypothalamus, amongst other midbrain areas, in response to pleasant oral milkshake stimuli correlates with BMI change after one year, with smokers also exhibiting significantly greater hypothalamic responses than non-smokers (Geha, Aschenbrenner, Felsted, O'Malley, & Small, 2013), thereby implicating the hypothalamus in both craving and feeding behaviour. Imaging the neural responses to fat content in pleasant vanilla-flavoured or strawberry-flavoured dairy stimuli indicated that fat is represented by BOLD activation in the lateral hypothalamus, amongst other regions (Grabenhorst, Rolls, et al., 2010). Specifically, the BOLD responses in the lateral hypothalamus correlated with the pleasantness ratings of the flavour ingested, echoing the increased firing rates of hypothalamic neurons with palatable stimuli (Fukuda et al., 1986).

1.4.4.4 – Proposed function and open questions

While the hypothalamus performs myriad regulatory functions within the human body, within the context of food intake and reward, the lateral hypothalamus appears to be implicated heavily in appetite regulation. Neural responses during ingestion of palatable foods and cessation of feeding, as well as connectivity to other parts of the limbic system such as the amygdala, indicate that the hypothalamus signals basic information about the stimulus to limbic reward areas that then evaluate these signals before sending them back to the hypothalamus, as evidenced by the link between hypothalamic activation and perceived pleasantness of oral food stimuli. Due to its anatomy and cytoarchitecture, it is likely that the hypothalamus would signal very basic information such as the caloric load of the ingested food item, which would then form part of the reward value of the food item. Subsequently, this value is reinforced through its connections with other parts of the reward system, which informs the hypothalamus of how adaptive, or maladaptive, the consumption of said food item would be, thereby allowing it to regulate its intake.

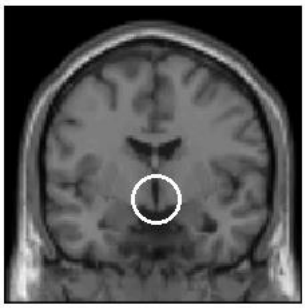

Figure 1.6. Region of interest for hypothalamus

1.4.5 – Amygdala

1.4.5.1 – Anatomy and connections

The amygdala forms part of the limbic system, located in the temporal lobe and just below the uncus (RajMohan & Mohandas, 2007). The amygdaloid complex is comprised of around 13 nuclei, all of which are distinguished based on their connections, histochemistry and cytoarchitecture (Krettek & Price, 1978). The amygdaloid complex can be roughly separated into three groups, namely the basolateral complex, the cortical complex and the centromedial complex (Müller & O'Rahilly, 2006; Sah, Faber, De Armentia, & Power, 2003).

The amygdala receives many projections from other areas in the limbic system and several cortical structures. Most pertinent to feeding behaviour, the amygdala receives inputs from the insular taste cortex and the hypothalamus. The medial forebrain bundle extends from the hypothalamus through the amygdala into other reward areas such as the ventral striatum and nucleus accumbens (You et al., 2001), which provides the amygdala with autonomic and visceral information. Cortical and thalamic inputs provide sensory information to the amygdala, with the greatest proportion of sensory information arising from the cerebral cortex (McDonald, 1998). These glutamatergic projections reach the amygdala primarily from layer V pyramidal neurons (Amaral & Insausti, 1992). Most notably, the amygdala does not tend to receive direct input from SI, such that most somatosensory information would reach the amygdala through the dysgranular parietal insular cortex instead (Shi & Cassell, 1998a). Gustatory information, on the other hand, appears to arrive from the insular taste cortex (Shi & Cassell, 1998b). Efferent outputs of the amygdala include the magnocellular mediodorsal thalamic nucleus (MDmc), which then projects to the orbitofrontal cortex (Timbie, García-Cabezas, Zikopoulos, & Barbas, 2020).

1.4.5.2 – Functional properties of single neurons

The amygdala, like most other structures within the limbic system, performs a wide variety of functions, ranging from emotions and fear to reward processing. However, within the scope of this thesis, the role that we will focus on is that of reward processing, specifically in the context of food intake. Amygdalo-hypothalamic circuitry is implicated in the learning of cues that override satiety, thereby promoting hyperphagy (Petrovich, Setlow, Holland, & Gallagher, 2002). Notably, lesioning the central nucleus of the amygdala results in loss of motivational arousal (J. Hall, Parkinson, Connor, Dickinson, & Everitt, 2001; Holland & Gallagher, 2003), implying a double dissociation between the different regions of the amygdala (Balleine, 2005). Similarly to the insular taste cortex and the OFC, the amygdala also encodes orally delivered stimuli (Kadohisa, Verhagen, & Rolls, 2005), although unlike in the OFC amygdalar responses show differential encoding primarily based on the viscosity of the oral stimulus, before then differentiating based on affective value (Kadohisa, Rolls, et al., 2005). Therefore, the role of the amygdala in the regulation of feeding, especially when accounting for learning, is intricate.

Like other reward structures, the amygdala responds to reward-predicting stimuli, although Bermudez and Schultz (2010a) demonstrated that the firing rates in the primate amygdala encodes the value of the reward in relation to the background reward, such that raising the value of background rewards extinguishes amygdalar responses (Bermudez & Schultz, 2010b). Notably, amygdala neurons also respond to the expectation of an incoming reward, displaying ramping behaviour similar to that observed in other reward structures (Belova, Paton, Morrison, & Salzman, 2007; Bermudez, Göbel, & Schultz, 2012). Reward encoding in the amygdala seems to be multisensory, wherein the sensory modality of the stimulus involved appears to be encoded in the specific pattern of the spike train (Morrow, Mosher, & Gothard, 2019). As a result, one would expect the amygdala to aid in the encoding of the rewarding properties of fat.

1.4.5.3 – Evidence from functional neuroimaging

In the context of reward and food intake, fMRI studies have implicated the amygdala in the encoding of the intensity of rewards, independent of the valence of the stimulus (Garavan, Pendergrass, Ross, Stein, & Risinger, 2001; Small et al., 2003). However, when the intensity of the reward is held constant, the amygdala appears to encode the valence

of the reward (Jin, Zelano, Gottfried, & Mohanty, 2015). Therefore, there appears to be an integrated intensity-by-valence interaction that corresponds to amygdalar activation, with positive and negative stimuli displaying differential encoding whereas neutral stimuli do not (Winston, Gottfried, Kilner, & Dolan, 2005). Specifically within the context of taste stimuli, the amygdala has been shown to respond to the taste of glucose (Francis et al., 1999), whereas this activation is significantly less prominent than that elicited by the taste of sodium chloride (O'Doherty et al., 2001). Amygdalar encoding of oral food stimuli also seems to be macronutrient-specific, as amygdala activations are recorded to correspond to the fat content of the oral food stimulus instead of the affective value (Grabenhorst, Rolls, et al., 2010). More recently, activation in the amygdala has been shown to be involved in planning food intake, where amygdalar activation predicts subjective internal saving plans for food intake, encoding both the value and the length of different future food choice outcomes with liquid food rewards being paid out as the stimuli (Zangemeister, Grabenhorst, & Schultz, 2016).

1.4.5.4 – Proposed function and open questions

Taken together, the evidence points to the role of the amygdala in encoding the presence of fat in the oral cavity (Grabenhorst, Rolls, et al., 2010), anticipatory reward planning of food intake (Zangemeister et al., 2016) and encoding of the subjective value of the reward through an interaction between the intensity and the valence of the reward (Garavan et al., 2001; Small et al., 2003). As a result, one would expect that the amygdala responds to rewarding fat content in oral stimuli. However, as the exact neural mechanisms behind fat detection is still largely unclear, the question of how the amygdala would sense the fat content of oral fat stimuli remains to be answered, as the structures from which the amygdala draws the information regarding oral fat content have yet to be established.

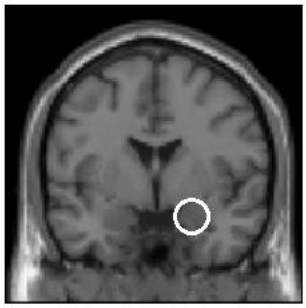

Figure 1.7. Region of interest for amygdala.

1.4.6 – Ventromedial Prefrontal Cortex/Pregenual Cingulate

1.4.6.1 – Anatomy and connections

The ventromedial prefrontal cortex (vmPFC) is a vast structure within the prefrontal cortex, widely implicated in a variety of cognitive functions. Due to the sheer volume of the structure and its involvement in such a large number of higher-order functions, this thesis will mostly focus on the overlap between this structure and the pregenual anterior cingulate cortex (pACC), which would encompass Brodmann's areas 24, 25 and 32, as well as some parts of area 10 due to direct inputs (Brodmann, 1914; E. T. Rolls, 2019). The vmPFC/pACC area is bordered by the callosal sulcus, located just anterior of the corpus callosum. The general ACC region is agranular and has a prominent layer Va, although area 32 specifically is notable for its dysgranular layer IV and densely pyramidal layer IIIc (Vogt, Nimchinsky, Vogt, & Hof, 1995).

This region of the pACC/vmPFC receives direct inputs from the OFC, as area 25 is known to receive direct afferent projections from the orbitofrontal areas 11 and 14 in addition to the frontal areas 46 and 9 (Vogt & Pandya, 1987). Area 46 also projects to area 24 in the ACC, which also has intra-cingulate reciprocal projections with area 25. Furthermore, amygdalar injections result in dense labelling of cells in areas 24 and 25. In general, it appears that the cingulate cortex receives inputs from visual, auditory and multimodal sensory areas, as well as premotor areas. In terms of efferent projections, area 32 differs substantially from areas 24 and 25 in that it projects mostly to medial and lateral orbitofrontal areas, whereas areas 24 and 25 project to more autonomous structures such as the premotor areas (Pandya, Van Hoesen, & Mesulam, 1981). This implies that final executive decisions on action plans are formulated in the vmPFC/pACC.

1.4.6.2 – Functional properties of single neurons

Single-neuron recordings in areas 24, 25 and 32 have shown that these regions are involved heavily in, amongst other functions, the encoding of value. Although the OFC is also implicated in value encoding (Padoa-Schioppa & Assad, 2006), value encoding in the vmPFC/pACC is distinct in that the encoding in this region is more sensitive to internal cues such as satiety, spontaneous initiation of action or thirst (Bouret & Richmond, 2010). Indeed, vmPFC stimulation on sated monkeys can elicit drinking behaviour (B. W. Robinson & Mishkin, 1968), implying its role in intake regulation. When presented with two options, vmPFC neuron responses reduced in proportion to the value and uncertainty associated with the two options and, subsequently, signal the chosen outcome, thereby implying mutual inhibition between neurons encoding the two options until one outcome is decided (Strait, Blanchard, & Hayden, 2014). In this regard, the function of vmPFC is distinct from that of the OFC, which appears to integrate value signals from different sensory modalities, in that it specialises in value reward comparisons. Furthermore, the direct efferent projections from the vmPFC to premotor areas imply that it is indeed the final step in decision making before an action is selected.

In addition to reward and uncertainty, ACC neurons have also been shown to encode punishment. Specifically, there is a population of neurons in the ACC that encodes the valence-specific value and uncertainty of rewards and another population that signals both reward and punishment (Monosov, 2017), implying that the ACC contains several distinct circuits that are able to process control-related and motivation-related signals to enhance behaviours that are valence-specific, such as avoidance or approach, or valence-neutral, such as vigilance. Therefore, the vmPFC/pACC area would be highly involved in the context of eating regulation, as it integrates internal and external cues to then form a subjective value of the food item in order to make choices.

1.4.6.3 – Evidence from functional neuroimaging

As with the single-neuron recordings in animal studies, human neuroimaging studies have also implicated the vmPFC as a region that encodes value. An advantage of using humans in the assessment of value is that they are able to perform more incentive-compatible tasks such as the BDM auction task (Becker et al., 1964) without an extensive instruction and training period. Plassmann, O'Doherty and Rangel (2007) used fMRI scanning on participants performing either a free-choice version of this task or a forced

version of this task (where the choice is made for them) in order to truly highlight the brain structures that encode subjective value by controlling for areas that are already active during economic choice but not necessarily correlating with subjective value. In doing so, they reported that BOLD activations in the dorsal ACC, in addition to the medial OFC and dorsolateral prefrontal cortex, were encoded the subjective value of participants. Notably, the value signals in the vmPFC corresponds to the subjective value as seen during real-life purchasing decisions, regardless of the category of goods, be they food, non-food consumables or gambles (Chib, Rangel, Shimojo, & O'Doherty, 2009), indicating that goal-directed value signals in the vmPFC are based on a common currency across which different choices can be compared, where the number of options does not appear to have an effect on ACC activations (Forstmann, Brass, Koch, & Von Cramon, 2006). Earlier studies showed that, while OFC activations are greater in forced-choice tasks, choices made of participants' own volition elicited greater activations in the ACC, with even greater activation on successful outcomes, implicating the ACC in the monitoring of task outcome (Walton, Devlin, & Rushworth, 2004). More pertinent to the role of reward valuation, while the OFC has identity-specific population-based value signals, the vmPFC encoding of value is identity-general, in that value signals can be decoded irrespective of the identity of the stimuli (Howard et al., 2015), where these signals show functional coupling with the amygdala, whereas identity-specific OFC signals are coupled with the relevant sensory cortex (in the case of olfactory stimuli, the piriform cortex). These findings indicate the role of the vmPFC/pACC in reward and punishment valuation.

1.4.6.4 – Proposed function and open questions

Taken together, these findings imply that, in the context of reward valuation, the vmPFC/pACC processes the value signals from the OFC into a common currency with which the choices are evaluated and selected. The sensitivity of vmPFC neurons to reward, punishment and uncertainty indicates that many complex processes occur in the vmPFC which unites all known information on the options before making a choice. In the context of oral food stimuli and eating behaviour, this would mean that the vmPFC receives the value signals of the oral stimulus after it has been collated into a coherent signal from the different sensory modalities. However, it is still not known if the value signals in the vmPFC can be modulated by changing OFC signals that feed into it through textural changes in oral food stimuli.

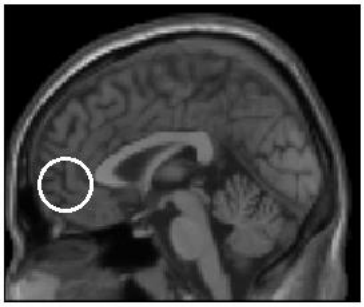

Figure 1.8. Region of interest for ventromedial prefrontal cortex/pregenual cingulate.

1.5 – Aims

This thesis aims to explore the function of the specific areas listed in Table 1.1, in addition to the mechanism of nutrient-specific sensing within them as well as their integration into a reward value representative of the food item. Further to elucidating the process of nutrient detection and reward valuation, we also aim to shed light on any connection between formally assessed economic value and their neural correlates with real-life eating behaviour.

Table 1.1

Relevant Structures and Their Known Functions in Food Processing

Region	Known Function
Anterior Insula	Primary taste cortex, responding to chemical and textural properties of oral stimuli
Posterior Insula	Somesthetic processing of oral stimuli, encoding textural properties such as viscosity
Orbitofrontal Cortex	Processing of stimuli from various sensory modalities, including taste and oral texture, to integrate them into a cohesive reward value
Oral Somatosensory Cortex	Responsive to somesthetic information within and around the oral cavity, responsive to fat content of oral stimuli through textural properties such as viscosity
Lateral Hypothalamus	Appetite regulation, encouraging eating behaviour and processing visceral stimuli to drive eating and stop feeding when satiety is reached
Amygdala	Reward processing of stimuli from various sensory modalities, including taste and oral texture, planned pre-prandial behaviour
Ventromedial Prefrontal Cortex/Pregenual Cingulate	Reward valuation, receiving reward signals from areas in the reward system to formulate a decision

1.5.1 – Hypotheses

This thesis will address and test the following hypotheses:

- The nutrient (fat and sugar) content of oral food stimuli is sensed through a combination of taste and textural properties.
- The economic value of food stimuli is determined by the nutrient content, which in turn is detected by the psychophysical properties of the stimulus.
- Distinct neural signals reflect specific nutrients based on sensory food properties and their economic valuation.

1.5.2 – Thesis Outline

This thesis will begin by exploring the optimisation process used to determine the experimental design of the project. A large proportion of the first year of the project involved optimising the stimulus set in order to produce a nutrient-controlled stimulus set that served to answer the fundamental biological questions but also retained the rewarding nature of pleasant stimuli. The subsequent chapter will then explore the psychophysical components of the stimuli, performing in-depth behavioural modelling to

examine the role of different psychophysical ratings in nutrient detection and determining reward value. The following chapter will then explore the neural correlates of these behavioural valuations, in specific brain structures, measured with fMRI, as well as comparing the subjective ratings and objective parameters for the stimuli. Finally, a discussion synthesising the findings and discussing them in the context of relevant literature will form the closing chapter of the thesis.

Chapter II – Optimisation and Data Acquisition

2.1 – Design of materials

2.1.1 – Design of primary stimuli

The design of the study requires that a set of stimuli with well-defined nutrient compositions be given to participants as they undergo fMRI scanning. In order to determine the specific effect of fat and that of sugar, it is crucial to create a set of stimuli that would have well-controlled fat and sugar levels. In addition, the stimuli should ideally be in liquid form to allow consumption during fMRI scanning, as it should be easily delivered to the participants and minimise their head movement during scanning. To that end, milkshakes were deemed to be the best form of liquid oral stimuli, as they allowed the natural modulation of various macronutrient levels. Milkshakes come with various macronutrient compositions, such that high-sugar, high-fat and high-protein milkshakes would still be relatively familiar to most participants.

In order to establish the effects of both sugar and fat, in addition to any combined effects, on the reward system, it was necessary to craft stimuli with varying levels of these two macronutrients. Therefore, a 2×2 factorial design was developed, where there were two distinct levels of fat and two of sugar, leading to 4 base stimuli (Table 2.1). These two distinct levels are clamped at certain amounts, such that the both low-sugar stimuli have the same concentration of sugar whereas both high-sugar stimuli also share the same concentration. The same is done with the modulation in fat content. This design allows the entire factorial dataset to be used in classifier analysis. For example, to run a classification analysis regarding sugar levels, both the low-sugar stimuli can be used as low-sugar data and both the high-sugar stimuli can be used as high-sugar data. In addition, this also allows a comparison of the difference between increasing macronutrient levels individually and increasing both fat and sugar levels simultaneously, as comparing the low-fat low-sugar (LFLS) against the high-fat high-sugar (HFHS) stimuli highlights this contrast.

Table 2.1.

Two-tiered Fat and Sugar Levels in Factorial Stimuli

	Low Sugar	**High Sugar**
Low Fat	Low-Fat, Low-Sugar (LFLS)	Low-Fat, High-Sugar (LFHS)
High Fat	High-Fat, Low-Sugar(HFLS)	High-Fat, High-Sugar (HFHS)

2.1.2 – Design of control stimuli

Control stimuli help dissociate certain nutrient effects from other physical characteristics. In the context of fat, two prominent properties come to mind, namely the viscous and lubricating properties of fat. Fatty foods tend to be significantly more viscous than their non-fatty counterparts. Furthermore, fat also tends to affect mouthfeel by lubricating food such that it slides more easily, thereby reducing friction between food particles and the mouth and the gullet and making mastication and swallowing easier. This property, known as the sliding friction coefficient, is often cited to be a driving force for the hedonic nature behind the consumption of fatty foods.

In the interest of establishing exactly how fat is sensed in the brain, and how it is eventually represented with a reward value, it is important to dissociate the signals associated specifically with fat and those pertaining to its textural properties, such as viscosity and sliding friction. One method of doing so would be to introduce fat-free stimuli that mimic these properties. Carboxymethyl cellulose (CMC) is a fat-free plant-based thickener that is often used in the food industry to increase the viscosity of low-fat and fat-free foods. As a result, it has also been used in several studies in order to increase the viscosity of stimuli without increasing its fat content.

The milkshakes used in the factorial design are also made by varying the concentration of milk and cream. Fat is introduced to the milkshakes in the form of single cream (Sainsbury's). This requires that all the fat present in the factorial design are derived from animal origin. It would be interesting to see how dairy fat differs from fat of plant origin, which can easily be done through the use of soya-based cream replacement (Alpro single cream). With a similar content of fat per 100g as the single cream used for the high-fat stimuli, soya cream allows a direct comparison of vegetable-derived fats and animal-derived fats.

Alongside fat and carbohydrates, protein is a crucial macronutrient for humans. Given that the recommended daily intake of protein is at least 0.8 g per kg of body weight, it stands to reason that there should be some reinforcement circuitry in the reward system to regulate its consumption. In order to examine if the reward encoding in areas such as the OFC is tailored to fulfilling calorie requirements or if it takes into account the nutrient components of food, having a high-protein stimulus allows us to examine the

potential difference in encoding of all three macronutrients. In addition, a high-protein high-sugar stimulus could also be used to examine the nature of superlinearity in fat and sugar encoding. Having protein instead of fat in this stimulus would allow us to see if the relationship between fat and sugar is exclusive or if this relationship also extends to other macronutrient pairings.

As fat and sugar have different caloric properties (sugar has 4kcal/g and fat has 9kcal/g), it would not be feasible to clamp the calorie content without adding too much sugar to the stimuli. If the high-fat, low-sugar (HFLS) and the low-fat, high-sugar (LFHS) stimuli were isocaloric, a proportionally larger amount of sugar would need to be added to the high-sugar stimuli. Therefore, only certain macronutrient levels were clamped such that all high-fat stimuli had the same concentration of fat and high-sugar stimuli had the same concentration of sucrose.

As the fat content of the stimuli in the factorial design was provided by dairy single cream, the notion of replacing dairy single cream with a plant-based cream substitute became of interest. This was due to non-dairy cream having a high-fat percentage from plant sources as well as having CMC added to increase palatability. Therefore, the physical parameters are slightly different than that of dairy cream and would be able to provide a better insight into how exactly physical parameters are involved in fat detection.

2.1.3 – Testing and refinement of stimuli

In the beginning of the study, the first half of experiments did 42 psychophysical ratings of the stimuli, whereas the second half did 42 BDM trials, such that participants received a separate briefing on how the BDM task works, which was deemed to be complicated. However, after the first 4 participants, the BDM task was integrated into the rating task as participants demonstrated good understanding of the task without requiring a second briefing in between the two tasks. The first set of stimuli featured the stimuli presented on Table 2.2. Fat content was modulated solely using single cream, that is, the fatty stimuli were pure single cream and the non-fatty stimuli were skimmed milk. Sugar content was modulated by adding sugar to the skimmed milk or the single cream, such that the low level of sugar was 0g of added sugar and the high level was 10g. (see Table 2.2)

The amount of fat and sugar that the participants received appeared to be excessive, to the extent that they became aversive. Figure 2.1 shows the averaged responses of the first 4 participants' (two female) subjective ratings of the stimuli as well as an example participant. The tendency towards selecting extreme values (either 0 or 10) increases with the number of trials, indicating either a heightened sensitivity to the stimuli after receiving such high doses of sugar and fat or reduced engagement in the task due to the aversive stimuli.

In addition to the ratings, one participant also asked for the experiment to be ended during the bidding task as they found the stimuli unbearably sweet and creamy. This led to a refinement of the stimuli, where a set of stimuli were tested such that they were tolerably creamy and not overly sweet, while still containing the different levels of fat and sugar that are crucial to the study.

Table 2.2.

Ingredients for 300ml of Stimulus Set 1

Stimulus	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Single Cream (ml)	300	0	0	300	0	0	0
Skimmed Milk (ml)	0	300	300	0	0	0	0
CMC (g)	0	0	0	0	3	6	6
Sucrose (g)	0	0	10	10	0	0	10
Total Sugar (g)	6.3	15	16.3	25	0	0	10
Total Fat (g)	54	0	0	54	0	0	0
Total Protein (g)	9.9	10.8	10.8	9.9	0	0	0

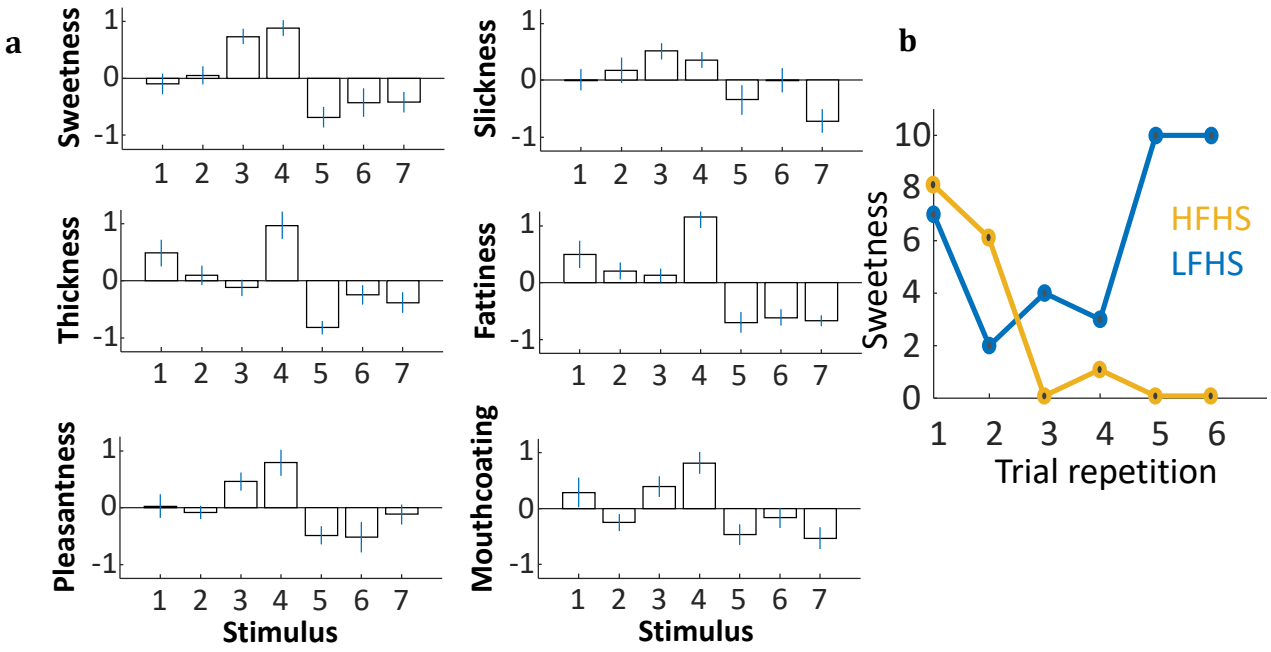

Figure 2.1. a) Z-scored normalised data of the basic psychophysical ratings from 4 participants. **b)** Trial-by-trial progression of sweetness ratings to two example stimuli, showcasing the tendency for extreme values (0 and 10) as the experiment progresses.

Two problems from the first stimulus set became apparent. Firstly, the stimulus set used unadulterated single cream as the high-fat stimulus, and the high-fat high-sugar stimulus was single cream with 10g of sugar. The high fat level of single cream (18g of fat in 100g of single cream) may have led to the fat content of the stimulus appearing aversive. The second problem that became apparent was that the first set of stimuli did not take into account that skimmed milk already contains a high level of simple sugars in the form of lactose (5g of sugar per 100ml). Therefore, adding an extra 10g of sugar into the low-fat stimulus, when the stimulus already contained 15g of sugar, was excessive. Furthermore, this meant that the HFHS stimulus contained less sugar than the LFLS stimulus. To that end, a new set of stimuli was developed where the low-sugar level was 15g of sugar in 300ml, and the high-sugar threshold was 20g of sugar, as seen in Table 2.3. This level could not go below 15g as the amount of lactose already present within skimmed milk was 15g/300ml. Furthermore, the single cream was diluted by adding skimmed milk to mitigate the fat content of the high-fat stimuli, such that the stimuli were a series of different fat concentrations. As pure single cream was deemed too fatty, the highest fat content was two-thirds single cream and one-third skimmed milk, whereas the medium fat stimulus was one-third single cream and two-thirds skimmed milk.

Table 2.3.

Ingredients for 300ml of Stimulus Set 2

Stimulus	1	2	3	4	5	6	7
Single Cream (ml)	200	200	100	100	0	0	0
Skimmed Milk (ml)	100	100	200	200	300	300	150
CMC (g)	0	0	0	0	0	0	6
Sucrose (g)	5.8	10.8	2.9	7.9	0	5	12.5
Total Sugar (g)	15	20	15	20	15	20	20
Total Fat (g)	36	36	18	18	0	0	0
Total Protein (g)	10.2	10.2	10.5	10.5	10.8	10.8	5.4

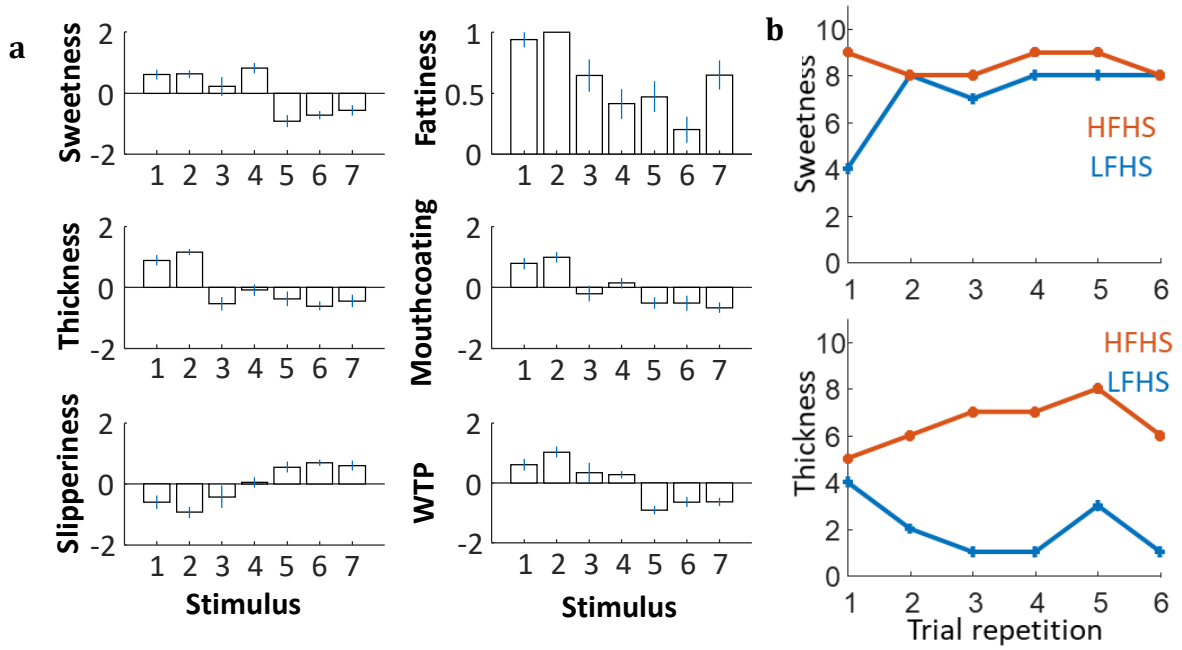

Figure 2.2. a) Z-scored data of ratings across 5 participants using the second set of stimuli. Notably, there is little discernible difference between HFLS and HFHS stimuli in sweetness as well as the disparity in sweetness between LFHS and HFHS. **b)** Sweetness and thickness ratings of certain stimuli as the experiment progresses. Much more stable ratings and more granularity observed in both rating scales.

Figure 2.2 shows the results of 6 participants with this stimulus set. Notably, the dilution of the single cream with skimmed milk, even at the highest fat level, rescued the

tolerability of the stimulus. As shown by the on-line ratings of the HFHS and LFHS stimuli, there is still an appreciable difference of thickness, in addition to more granularity of the ratings. However, there is a notable lack of difference between the high-sugar and low-sugar stimuli (as seen in the sweetness ratings of Stimulus 1 against Stimulus 2), possibly owing to a difference in perception between sucrose and the lactose present in skimmed milk. In order to rectify this, we developed a stimulus set where the base LFLS stimulus is equal parts skimmed milk and water, such that the macronutrient distribution is as shown in Table 2.4.

Table 2.4.

Ingredients for 300ml of Stimulus Set 3

Stimulus	1	2	3	4	5	6	7
Single Cream (ml)	200	200	100	100	0	0	0
Skimmed Milk (ml)	50	50	100	100	150	150	150
CMC (g)	0	0	0	0	0	0	6
Sucrose (g)	3.3	13.3	2.9	12.9	2.5	12.5	12.5
Total Sugar (g)	10	20	10	20	10	20	20
Total Fat (g)	36	36	18	18	0	0	0
Total Protein (g)	8.4	8.4	6.9	6.9	5.4	5.4	5.4

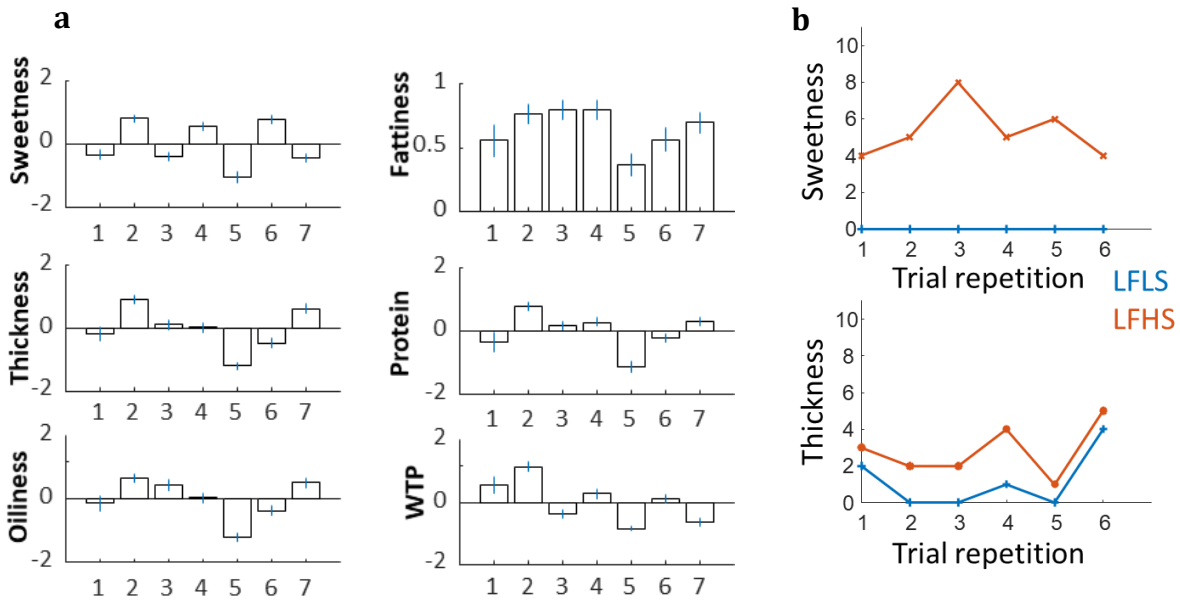

Figure 2.3. a) Z-scored data across 5 participants with Stimulus set 3. Note well the sweetness effect and the thickness effect highlight the increasing sugar and fat content, as expected. Furthermore, the bid values for the high-fat stimuli are still very high, indicating this level of fat is optimal. **b)** Trial-by-trial ratings of the low-fat low-sugar and low-fat high-sugar stimuli across sweetness and thickness scales, showing a stable distribution of ratings with meaningful differences.

Normalised results from Figure 2.3 show that there is a much more appreciable and marked distinction in sweetness between the high-sugar and low-sugar stimuli in this stimulus set. This is further reinforced by the example online ratings that show marked differences in sweetness between low- and high-sugar stimuli, as well as thickness differences between low- and high-fat stimuli, which is exactly the gradation that we expected. Furthermore, the WTP ratings of even the highest fat percentage is still very high, indicating that across participants the fat concentration used in the highest fat category was not aversive. Therefore, the highest fat level of 36g in 300ml was deemed the most appropriate for the high-fat stimuli in the final stimulus set, and the sugar level of the high-sugar stimuli capped at 20g and the low-sugar stimuli capped at 10g.

Having now established the stimuli used in the factorial design, we now turn our attention to the development of the control stimuli. The notion of using protein as a control stimulus for fat, in order to establish if the effects seen between fat and sugar is exclusive only to fat or if it can be generalised to other nutrients, became a primary concern. After procuring the pure whey protein powder (BULKPOWDERS Whey Protein Isolate 97), we needed to establish what level of protein to use in our stimulus set. As a

standard serving is 25-30g of the whey, we decided to have two protein levels, namely a half-serving (15g) and a full serving (30g), such that our stimulus set macronutrient breakdown can be seen in Table 2.5. We conducted tests on two participants using this stimulus set (with results in Figure 2.4), after which the half-serving was deemed to be the most appropriate for our study design, as the full serving of pure whey protein often led to agglomeration of the stimulus, which blocked the peristaltic pumps from functioning properly.

Table 2.5.

Ingredients for 300ml of Stimulus Set 4

Stimulus	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Single Cream (ml)	200	200	0	0	0	0	0
Skimmed Milk (ml)	50	50	150	150	150	150	150
CMC (g)	0	0	0	0	0	0	6
Sucrose (g)	3.3	13.3	12.9	12.9	2.5	12.5	12.5
Whey Powder (g)	0	0	15	30	0	0	0
Total Sugar (g)	10	20	20	20	10	20	20
Total Fat (g)	36	36	0	0	0	0	0
Total Protein (g)	8.4	8.4	20.4	5.4	5.4	5.4	5.4

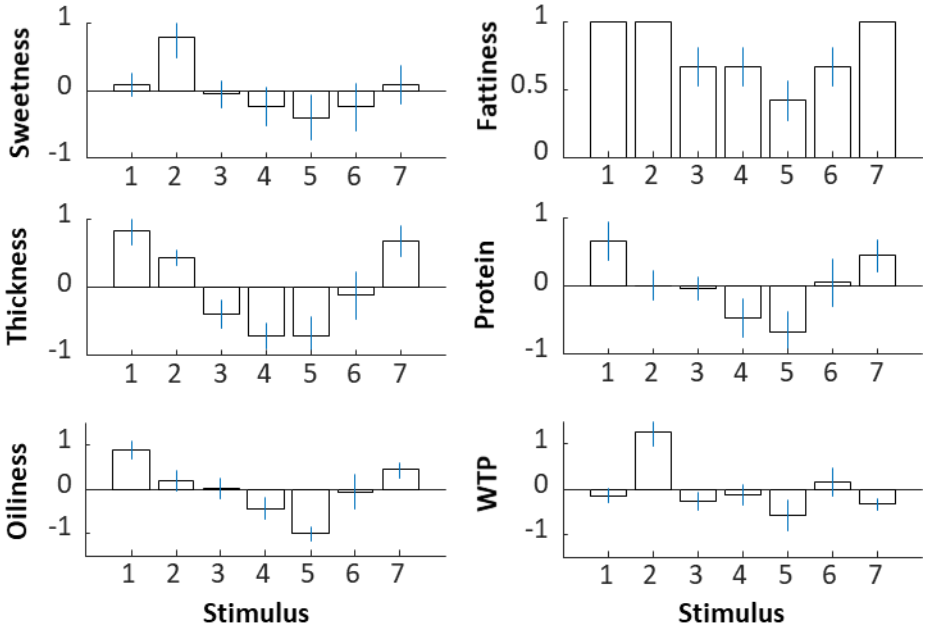

Figure 2.4. Z-scored normalised data across 2 participants. We then decided to go with the lower protein content as the higher protein content led to clumping of the stimuli and blockage of the pumps.

With the protein stimulus established, we also pondered about the selectivity of the fat stimuli, namely if reward responses to fat were specific to fat from dairy sources (single cream) or could also be generalised to fat from plant sources, which we investigated using a soya-based single cream replacement (Alpro UHT soya cream). As the fat content is only marginally lower than the fat content of the single-cream stimulus, similar methods were used for its dilution. The stimulus consists of two-thirds soya-based single cream and one third water and skimmed milk, with the required amount of granulated sugar added at the end to ensure that the stimulus had 20g of sugar per 300ml. The breakdown of ingredients and macronutrient content can be found in Table 2.6.

Table 2.6.

Ingredients for 300ml of Stimulus Set 5 (Final Stimulus Set used for fMRI)

Stimulus	1	2	3	4	5	6	7
Single Cream (ml)	200	200	0	0	0	0	0
Skimmed Milk (ml)	50	50	150	100	150	150	150
Soy Cream (ml)	0	0	0	200	0	0	0
CMC (g)	0	0	0	0	0	0	6
Sucrose (g)	3.3	13.3	12.9	12.6	2.5	12.5	12.5
Whey Powder (g)	0	0	15	0	0	0	0
Total Sugar (g)	10	20	20	20	10	20	20
Total Fat (g)	36	36	0	30	0	0	0
Total Protein (g)	8.4	8.4	6.9	6.9	5.4	5.4	5.4
Energy (kcal)	399.7	439.7	340.5	389.4	65.5	105.5	105.5

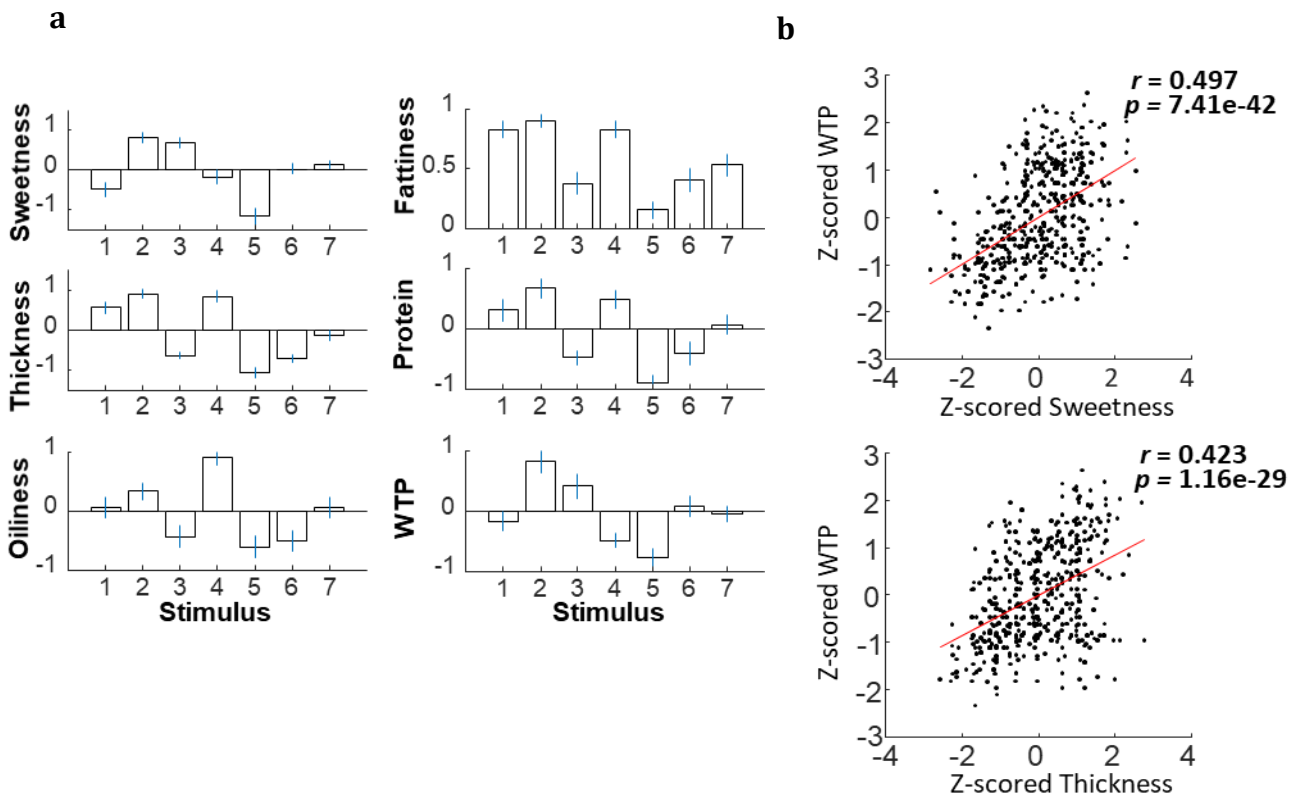

Figure 2.5. **a)** Z-scored ratings from 5 participants of Stimulus set 5**.** Noting the appreciable differences in the sweetness and thickness ratings, as well as the impact these qualities have on subjective value without being intolerable (**b**), we decided on this stimulus set as the final set.

Figure 2.5 shows the Z-score normalised data of the psychophysical ratings of this stimulus set across 5 participants. As visible across all the relevant psychophysical ratings, but most importantly those of sweetness, thickness, fat content and willingness to pay, this stimulus set elicits the intended effects. The differences in sugar level are reflected in the sweetness ratings and the variety in fat levels are reflected in fat content and thickness. Furthermore, the willingness to pay ratings indicate that subjective value increases along with sweetness and thickness, without breaking the correlation by being too sweet or too thick. Therefore, this stimulus set became the final stimulus set that is used throughout the rest of the experiment and in the scanning task.

In between the stimuli, the participant also receive a rinse solution composed of distilled water, sodium carbonate and potassium chloride in an ionic concentration similar to that of human saliva, which has been used in previous studies in the field (Grabenhorst, Rolls, et al., 2010; Seubert et al., 2015). This avoids activations in the taste

cortex that arise due to the presence of water in the oral cavity (De Araujo, Kringelbach, Rolls, & McGlone, 2003).

2.1.4 – Physical parameters of stimuli

2.1.4.1 – Viscosity

Once the stimulus set for the entire experiment had been finalised, we investigated the various physical parameters of the stimuli. As sugar is detected chemically through the activation of taste receptors, the concentration of sugar in the stimuli is the measured physical parameter for sugar detection. However, as fatty stimuli have very different textures to non-fatty stimuli, it was important to characterise the textural properties of the stimuli. To that end, we measured the viscosity and the CSF of the stimuli.

A rheological assessment of the stimuli allowed us to examine the viscosity of each stimulus. Viscosity is the measure of how objects flow, which can generally be thought of as having an extensional component (particles moving away from each other) and a shear component (particles sliding over and around each other). The measure of shear flow – that is, shear viscosity – is the pertinent aspect for food consumption, as the mastication of food to turn it into an easily-swallowed bolus in the mouth involves more the agglomeration of food particles that their separation.

The measurement of shear viscosity can be thought of by the measure of the shear stress required to move the uppermost layer of a liquid a distance of x while its bottommost layer a height of h away remains stationary. As greater shear stress (σ) is used, the shear strain (γ) which is the relationship of the distance x and the height h changes ($\gamma = \frac{x}{h}$). In fluids, the shear stain increases for the period of applied stress. Therefore, the rate of change of shear strain with respect to time is the rate of momentum transfer to the uppermost layer of the fluid ($\dot{\gamma} = \frac{d\gamma}{dt}$), also known as the shear rate. Shear viscosity (η) is the measure of the ratio between shear stress and the shear rate, leading to the equation:

$$\eta = \frac{\sigma}{\dot{\gamma}}$$

Shear viscosity can be measured in Pascal per second (Pa s^{-1}), although most common fluids that are encountered in the context of nutrition are measured in

centipoise (cP). Table 2.7 shows the viscosities of everyday fluids in cP, where notably the differences between common fluids tend to be in large orders of magnitude.

Table 2.7.

Viscosity Values of Common Fluids

Fluid	Water	Single Cream	Vegetable Oil	Glycerine	Maple Syrup
Viscosity (cP)	1	12	55	1490	3200

Within the current study, the rheometry of the stimuli was done with the help of the Department of Chemical Engineering at the University of Cambridge. The assessment was performed using the ARES G2 rheometer (TA Instuments, USA) with a cup diameter of 34.0mm, bob diameter of 32.0mm and a bob length of 34.0mm. Rotational rheometers such as the ARES G2 measure the shear viscosity of fluids using two parallel plates, between which the fluid is placed. The top plate is then able to either apply a controlled rotational torque and measure the resultant rotational speed or control the rotational speed and measure how much rotational torque is required to maintain it. In doing so, one is able to measure the resultant shear stress and the shear rate of the fluid, the ratio of which gives the shear viscosity of the fluid. In our case, we allowed the samples to reach the experimental temperature for 300 seconds and conducted shear rate sweeps from 100Hz to 0.1Hz or 1Hz. The measurements were performed at 10^{o}C, the temperature at which the stimuli are maintained during the experiment through the use of a cooler box and ice. The sweeps start by rotating the cup clockwise and subsequently anticlockwise, known as a two-way sweep measurement.

Some fluids display non-Newtonian behaviour, such that the ratio of the shear rate and the shear strain is not linear. Therefore, the lack of a constant derivative means that the viscosity of the fluid changes depending on the shear strain applied to it. One everyday example of this is a suspension of corn starch in water, as found in ketchup, where the shear viscosity of the fluid decreases under greater shear strain, leading to a nonlinear increase in flow rate with greater shear strain. This phenomenon is known as shear-thinning. While most of the stimuli tested here displayed Newtonian viscosity properties, shear-thinning can be observed in both the CMC stimulus and the soy protein stimulus. This is likely due to the CMC content, which is known to have shear-thinning properties.

In these cases, the value of the viscosity used is that displayed by the stimulus when the rotational rheometer is spinning at 50Hz in a clockwise direction. If these values differ after three measures, an average is taken. The viscosities of the stimuli in centipoise (cP) can be found in Table 2.8.

Table 2.8.

Viscosity Values of Each Stimulus

Stimulus	**HFLS**	**HFHS**	**Protein**	**Soya**	**LFLS**	**LFHS**	**CMC**	**Rinse**
Viscosity (cP)	8.86	12.64	3.07	68.2	2.15	2.26	59.26	1.4

2.1.4.2 Coefficient of sliding friction (CSF)

The other physical parameter of interest in this study, namely the lubricative nature of fat, can be quantified through the change in sliding friction between the palate and the tongue. This characteristic nature of fat is not shared with other macronutrients and is a putative mechanism by which the brain senses the presence of fat in a given food item. In fluids such as fat and oil, friction is often divided into internal friction and external friction. Internal friction is a measure of how easily fluid particles move among each other, which is essentially a measure of viscosity, whereas external friction describes the relationship of these fluid particles with other matter – that is, the cohesion between fluid particles and the other surfaces they come into contact with such as the palate and the tongue. It is this external friction that gives fat that lubricating nature.

The measurement of the coefficient of friction of the stimuli in the study was performed with the help of the Engineering Department at the University of Cambridge, with whom we collaborated to create a custom tribometer. As external friction is modulated by the adhesion between the fluid and the external material, it is crucial to recreate the biological oral environment as faithfully as possible. To that end, we measured the friction using two adjacent pig tongues as the surface of interest instead of using artificial materials such as plastic or metal. The base tongue was applied flat on an aluminium platform, whereas the mobile upper tongue-tip was attached to a hemispherical slider with a radius of 100mm. The slider is then mounted onto a track consisting of two rails to ensure that it follows a constant trajectory. Through the use of a counterweight, the weight of the tracks were balanced out such that the only weight acting on the contact point between the tongues was that of the slider and tongue-tip

(2.58±0.07N). The slider was then connected to the Instron 5544 Universal testing machine (Instron, USA) using a light string and a pulley.

In order to measure the CSF of our stimuli, a sample was applied liberally on the lower tongue base, after which the slider and tongue tip are applied. The Instron then moved the slider at a constant velocity (16 mm/s) along the base tongue. While velocity is constant, Newton's First Law of Motion states that the total force acting on the slider is 0, such that:

$$F = \mu N$$

Where F is the traction force applied by the Instron, N is the loading force perpendicular to the contact surface and μ is the coefficient of sliding friction. The equation can be represented as such:

$$\mu = \frac{F}{N}$$

Such that the CSF is effectively the ratio of the measured force applied by the Instron and the loading force.

Fresh pig tongues were obtained one day before testing from a local butcher (Leech & Sons, Royston, UK) and rinsed with water to remove blood and tissue fluids. The top 1cm layer of 18cm of the anterior tongue was then extracted to ensure that a flat surface would be fitted onto the testing platform. These slices were then preserved in an isotonic saline buffer (Phosphate-buffered saline, PBS, 1X, pH 7.4) in a freezer below 4°C overnight. On the day of testing, the tongue surfaces to be used for the base (18cm) were glued onto the base platform and another tongue tip (5cm) was attached onto the hemispherical slider. The slider and tongue tip were then weighed to calculate the loading force (N). Prior to each measurement, both tongue surfaces were rinsed with 10 mL of PBS three times to remove residual testing liquids and ensure that the tongue slices were sufficiently hydrated. 30mL of the liquid sample was then used to coat the base tongue, after which the Instron pulled the slider at a constant velocity from the base of the tongue in an anterior direction.

Three measures of each stimulus was obtained using, two pairs of pig tongues in opposite, namely both pairs of tongues were exclusive for testing purposes. This was done to cancel out the effect of the order to stimulus testing. For example, a high-fat

sample may leave residue even after rinsing due to the coating nature of fat. As there is a higher incidence of mechanosensory receptors towards the anterior of the tongue, we wanted to focus our analysis on the most anterior portion of the tongue possible. However, due to the shape of the tongue, the more anterior portions tended to be too thin to allow stable measurement. Therefore, the anterior 5-7 cm of the tongue was used as the region of analysis as it had the best overlap of these two criteria. Due to individual variations amongst tongues, the absolute values of the CSF naturally differed between the two pairs. In order to ensure this was comparable, the absolute value of the rinse solution was then used as the anchoring value, where it was normalised to 1 and all other values revised accordingly. The values across both tongues were then averaged to obtain the values used in our analyses, which can be found in Table 2.9.

Table 2.9.

Normalised Coefficient of Sliding Friction Values of Each Stimulus

Stimulus	HFLS	HFHS	Protein	Soya	LFLS	LFHS	CMC	Rinse
CSF	0.420	0.454	0.706	0.372	0.861	0.833	0.316	1.00

2.2 – Design of equipment and task

2.2.1 – Design of peristaltic pumps

In order to deliver the stimuli while the participant undergoes MRI scanning, peristaltic pumps are used. The pumps are set up in the scanner control room, where the experimenter, the radiographer and the testing laptop are. Silicone tubing is then connected to each pump output and threaded through the connecting aperture into the scanner room.

The design of the pumps themselves are based on a previous experiment where a small rotor presses food-grade tubing against the walls of the pump, creating a suction force that drives the liquid from the liquid input through to the output of the pumps. The signal input for each pump is from a modified plug terminal that connects to a National Instruments card (NI card, Texas, USA) connected to the testing laptop. When activated via MATLAB, the NI card applies a 5V potential difference on the signal input, causing the circuit to trip and causing the rotor to turn at a fixed rotational speed until the potential difference is removed, thereby allowing control of the length of time the pump runs for.

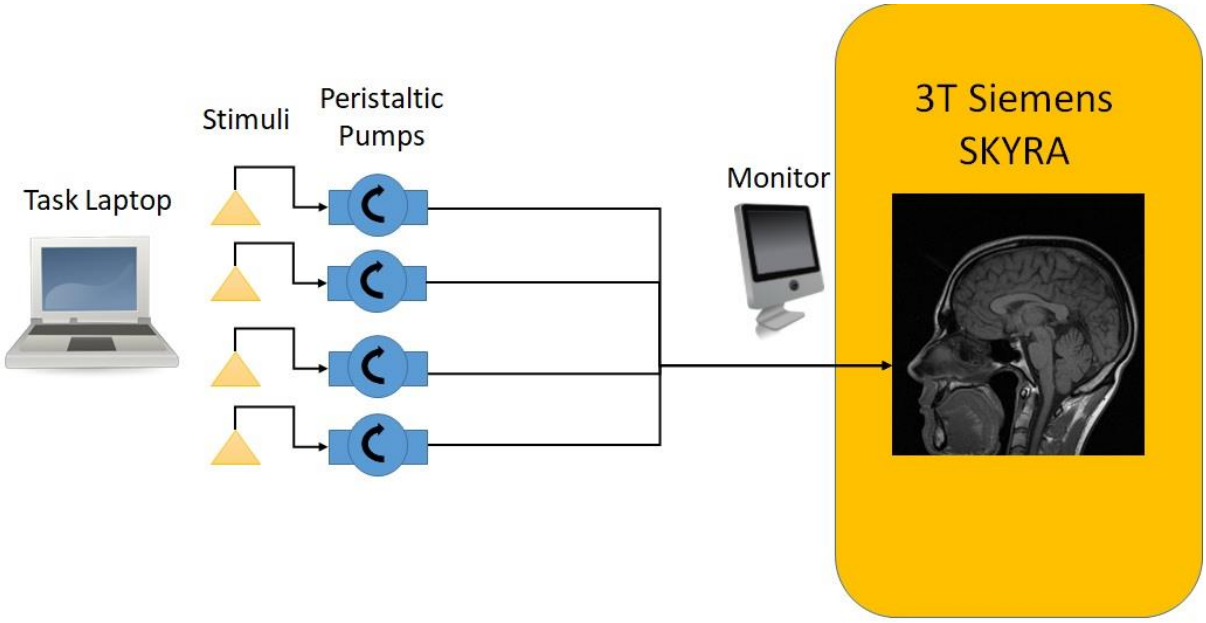

Figure 2.6. Diagram of pump and connections

2.2.2 – Calibration of pumps

As mentioned in ***2.2.1***, the rotational speed of the rotor is constant within each pump, depending on the structural integrity of the parts themselves. Therefore, we are only able to modulate the volume of the liquid delivered through modulating the length of time the pumps run. From previous studies involving liquid reward delivery in the fMRI, we aimed to deliver between 0.75ml and 1ml to participants as this is known to be enough to elicit activations in taste-processing areas such as the insular taste cortex.

As the pumps have different drawing capabilities, and as the physical parameters of the stimuli (such as viscosity, adhesion and cohesion) vary greatly, it was crucial to assign one stimulus to one specific pump and calibrate each stimulus to their respective pumps such that 0.75-1ml of the stimulus is delivered. Below are listed the amount of time in milliseconds that each pump runs to deliver the required amount of their respective stimuli.

Table 2.10.

Length of Opening Time for Each Pump

Stimulus	**HFLS**	**HFHS**	**Protein**	**Soya**	**LFLS**	**LFHS**	**CMC**	**Rinse**
Time (ms)	150	175	200	350	200	250	300	200

2.2.3 – Tongue movement

In order to ensure even dispersal of the stimuli and rinse, and to ensure that texture-related subjective ratings were not confounded by variability in tongue movement, participants were instructed in how to move their tongue during the tasting period. In the behavioural pre-testing task, a cursor appeared in the centre of the screen during the tasting and rinse period. This cursor would move either to the left, then to the right before coming back to the centre or to the right, then the left before coming back to the centre, and participants were asked to move their tongue across the palate mirroring the cursor's movement. The direction in which the cursor moved was randomised for every trial and taste period such that the participant could not predict the direction before the appearance of the cursor. This task trained the participants to move their tongues with a specific shear speed and reduced the likelihood of results arising from differences in tongue movement.

During the fMRI scanning task, however, the tongue movement cursor is replaced with an arrow. This replacement reduced the need for participants to track the cursor movement across the screen with their eyes in the hopes of minimising visual effects that are irrelevant to the processing of the oral stimuli. Furthermore, having already been trained on the required tongue movement speed, participants were likely to faithfully adhere to the trained speed.

2.3 – Behavioural Responses

2.3.1 – Psychophysical Ratings

As the experiment focuses specifically on putative pathways for nutrient detection, it was also crucial to examine how participants subjectively perceive the presence of different nutrients in the stimuli they receive. This would therefore require asking participants to rate the stimuli they receive on defined scales, as this would shed light on which properties of the stimuli are used in the detection of nutrients such as fat and sugar.

Naturally, sugar content is readily reflected by the perceived sweetness of the stimulus. As sugar is known to activate TIR2+TIR3 receptors in the mouth, which ultimately feed into the insular and opercular taste areas (G. K. W. Frank et al., 2008). As seen in the stimulus optimisation phase, we were able to reliably track sugar content

using a sweetness rating scale between 0 and 10. Hence, we maintained this scale in the final ratings.

Given the different proposed mechanisms of fat detection in the brain, the subjective perceptions of the physical parameters of the stimuli related to fat perception should also feature in the rating scales. Therefore, a thickness scale was also used in the study, where participants were instructed that a rating of 0 means completely watery and a rating of 10 means very thick. Notably, the thickness ratings for each stimulus correlates strongly with the log of the viscosity of each stimulus, indicating that the thickness ratings do indeed capture the variance in viscosity.

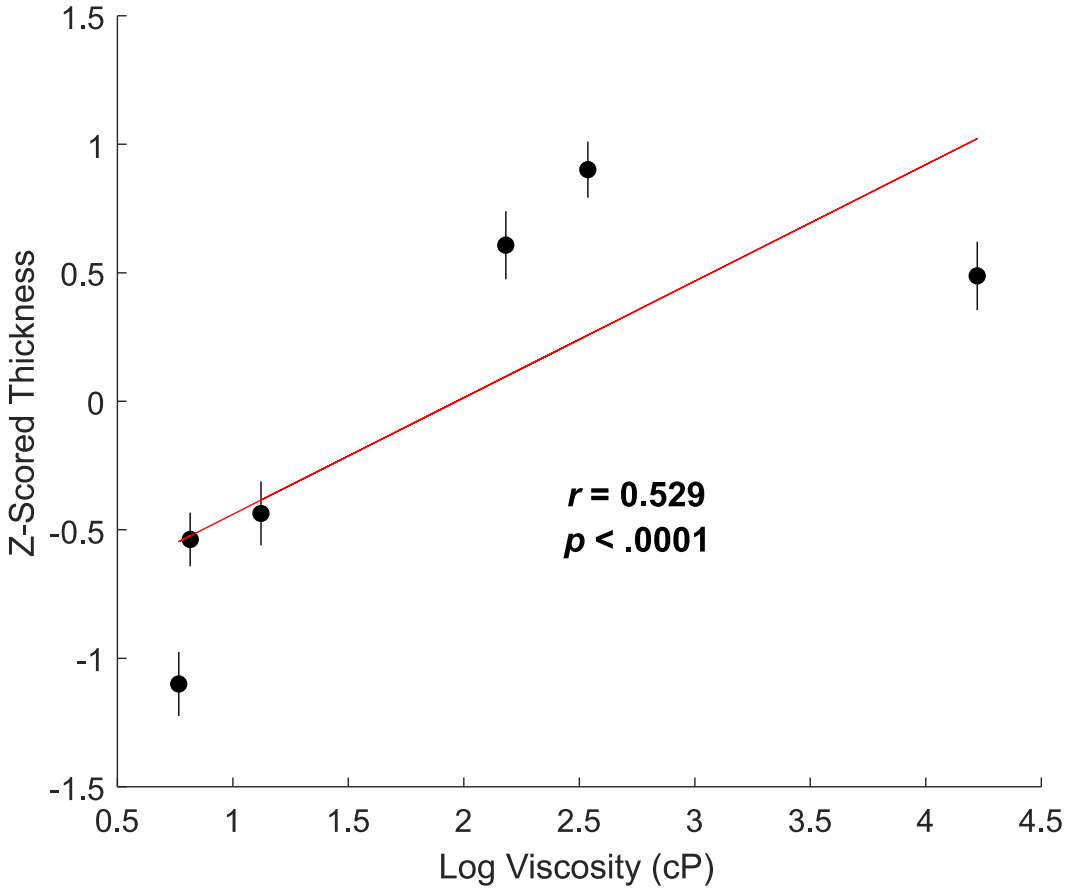

Figure 2.7. Correlation of viscosity and thickness ratings in 31 participants using the final stimulus set.

The other physical parameter related to fat detection is the coefficient of sliding friction. However, this proved a difficult concept to convey to most participants, as there was not a readily available everyday word that we were able to think of to convey this. At

first, we used the term 'slickness', which at first glance seemed to convey the lubricative nature of fat. However, this proved difficult as participants repeatedly asked about this rating scale in particular before and after the experiment. We then replaced it with 'slipperiness', which is not in itself a word that is often used to describe food. As a result, participants still struggled to apply the concept of how slippery something is to food stimuli. Finally, the term 'oiliness' was introduced as participants referenced that sensation during the pre-test briefing of the task multiple times. Notably, the oiliness rating of the stimuli does indeed correlate negatively with the coefficient of sliding friction. Therefore, the term 'oiliness' was maintained.

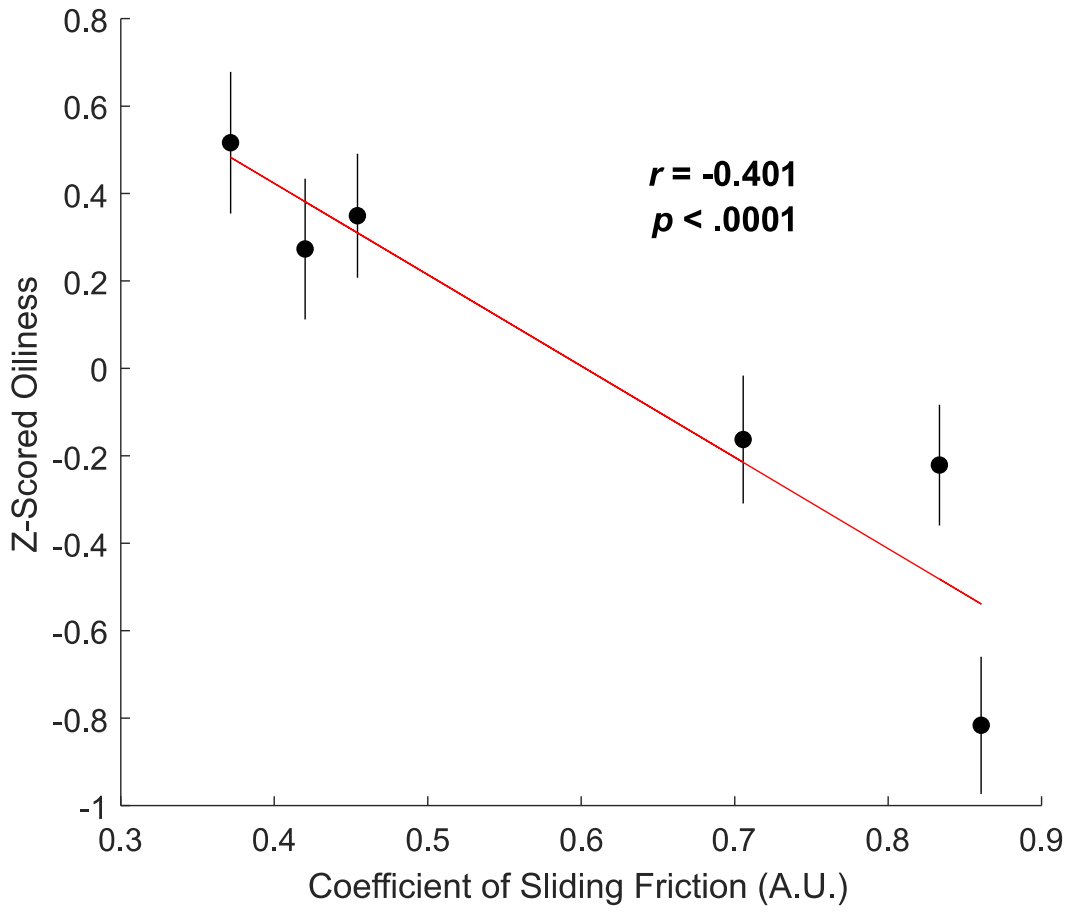

Figure 2.8. Correlation of oiliness and CSF in 31 participants using the final stimulus set.

Initially, a scale measuring the subjective report of the mouthcoating nature of the stimuli was trialled, featuring a scale of 0 to 10. However, this was eventually left as it did not have a relationship with any of the physical parameters that were measured.

Nevertheless, as we were still interested in the neural mechanisms of fat detection, it was important to also ask participants if they detected any fat in the stimuli. Therefore, a fat-detection scale was introduced where participants were asked if the stimulus contained any fat using a binary rating. This was then used as a measure of fat detection in participants.

A pleasantness scale was also introduced at the beginning of the experiment. This was, nevertheless, deemed superfluous, as we also used a Becker-DeGroot-Marschak (BDM) auction bidding task as a measure for subjective value (c.f. Section 2.3.2). Eventually, the pleasantness scale was replaced with a protein rating, where participants were asked how much protein would be present in a 250ml cup of the stimulus. The possible responses were 0g, 5g, 10g, 15g, 20g and 25g. As the protein contents of the stimuli were well controlled, and as there was also a stimulus that was specifically high in protein, this rating scale was useful to determine if humans are able to detect explicitly the protein content of food.

2.3.2 – Subjective Value

As the study focuses greatly on both the detection of various nutrients as well as how these detection signals are then integrated into a coherent value signal, measuring subjective value of the stimuli became a point of interest. This study features a modified version of the BDM auction task (Becker et al., 1964). In a traditional BDM task, a subject would place a bid for an item, which is then compared to a randomly generated computer bid. If the subject bid is higher than the computer bid, the subject wins and has to pay the required sum in order to receive the reward. If they subject's bid is lower, the subject loses the bid and does not have to pay the required sum but also does not receive the reward. This method has shown incentive compatibility, as participants must choose between the budget that they have and the amount they are willing to spend, thereby avoiding participants over- and under-spending on specific rewards. The resultant bid should therefore be a faithful reflection of the participant's subjective value of the reward offered in relation to the other rewards.

The BDM task, however, had to be modified slightly to fit the current experiment such that the participant does not receive a large payout of the stimuli after every trial. To that end, participants provided a bid of 0 to 10 credits on the stimulus they received. At the beginning of the behavioural pre-test, as well as at the beginning of each scanning

run, the participant receives 100 credits to spend on their bids, where each credit was worth 0.01p. As the pre-testing consists of 42 trials (6 trials of 7 stimuli in a randomised order), participants still have to budget their 100 credits across the 42 trials. They are also asked to use similar bidding strategies during the scanning runs. As in the BDM auction proper, when participants lose the bid, they lose none of their budget, although when they win the amount they bid is deducted from their budget. In order to maintain incentive compatibility, they are told at the beginning of the experiment that one of the bids they win would be randomly selected and they would receive a 250ml cup of the reward. Therefore, the participants were still incentivised to spend more on the stimuli that they enjoyed, a notion that was underlined in the pre-experimental briefing for each participant. Figure 2.9 shows the extent of the efficacy of these instructions, as there is a notable distinction of the willingness to pay (WTP) across the factorial stimuli, providing a ranking of subjective values of various stimuli. Notably, the stimulus with the highest fat and highest sugar content (HFHS) reliably ranked highest across all participants, indicating that it was the most valued stimulus.

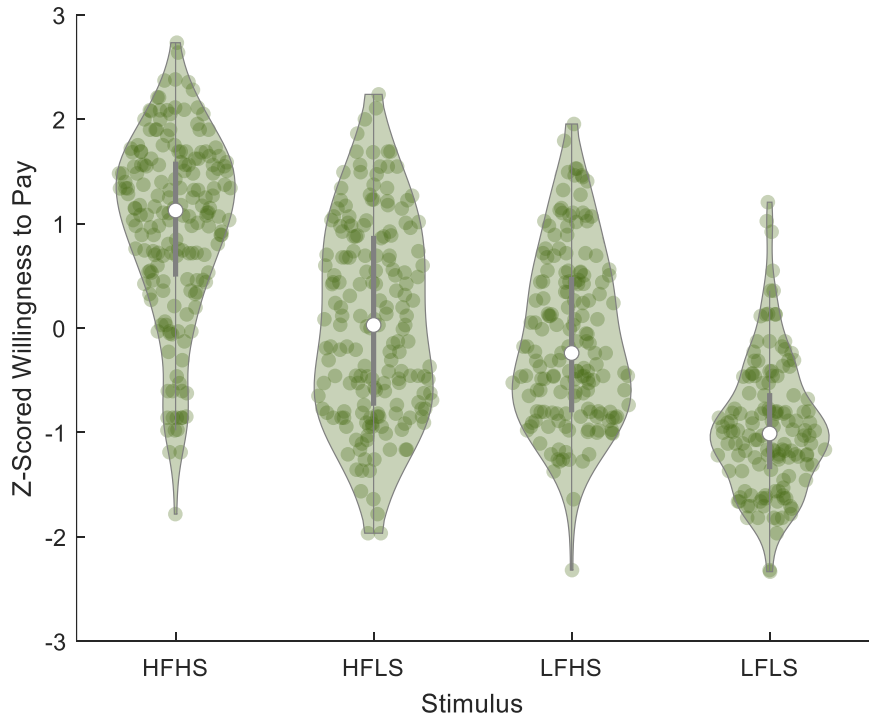

Figure 2.9. Z-scored normalised willingness to pay (WTP) across the experimental stimuli. Notably, across all participants, the HFHS stimulus had the highest WTP rating, the LFLS stimulus the lowest and the HFLS and LFHS in between.

2.4 – Pilot scanning

Pilot scanning was conducted on two participants before commencing the study proper. Initially, the setup involved 5 runs of 28 trials each, with each run lasting about 20 minutes. In both cases, participants elected to terminate the experiments during the third run of scanning due to issues that affected their ability to continue with the experiment.

2.4.1 – Refinement of mouthpiece

The initial mouthpiece used in scanning was identical to the one used in the behavioural pre-testing, which consisted of two layers of heat shrink of decreasing diameter placed consecutively to reduce the aperture through which the stimulus enters the mouth. This was deemed to work well in the behavioural pre-testing, and no issues arose from this. However, in translating it into the scanning environment, the supine position of the participant meant that this small aperture would, with the help of gravity, shoot strongly towards the back of the participant's mouth, a sensation which both pilot participants reported as exceedingly unpleasant. This was ultimately resolved by modifying a baby food feeder (Losuya, China), which is shaped similarly to an infant dummy with holes to allow the liquid to go through. By increasing the number of holes, we were able to ensure that all the stimuli would be available to the participant while its delivery is still well-dispersed in the oral cavity. Pre-testing with participants showed a positive response to using this mouthpiece to ingest pumped liquids while in a supine position. All scanning sessions thence used the modified mouthpiece.

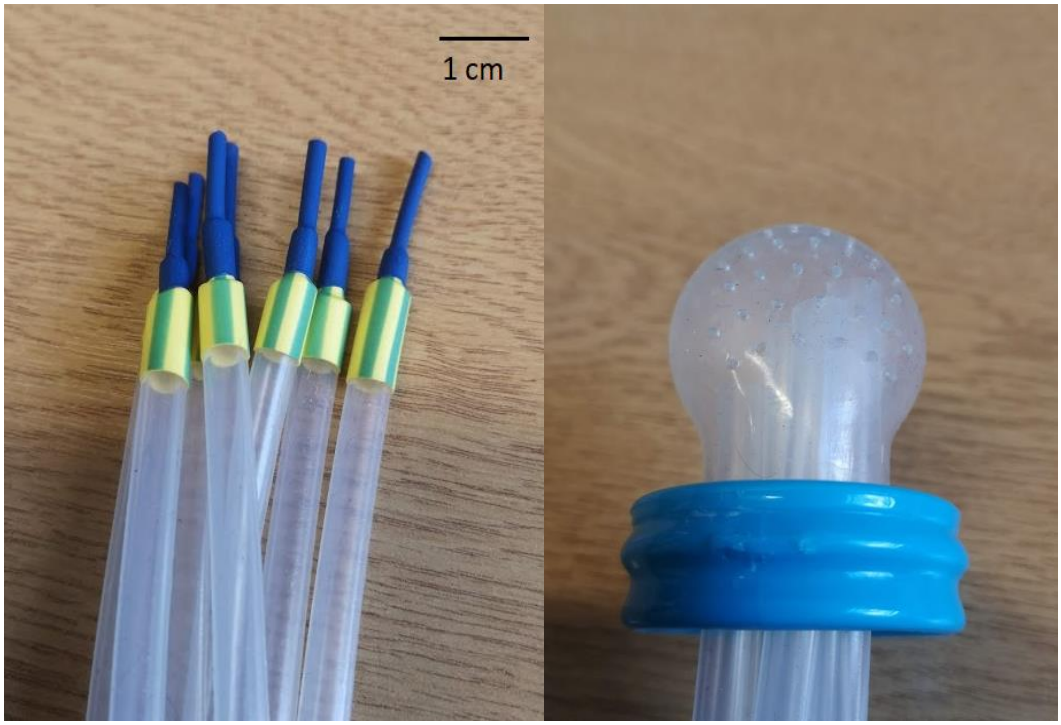

Figure 2.10. Picture of original heat-shrink mouthpiece and modified mouthpiece

2.4.2 – Refinement of rinse stimulus

The rinse solution used in the behavioural pre-testing was mineral water, as it only needed to rinse the oral cavity and it was readily available. However, water is known to activate the taste cortex in humans (De Araujo et al., 2003). To counter this, the rinse solution for the scanning component of the experiment was a solution largely isotonic to human saliva, commonly used in the field to avoid the activation of the taste cortex (Grabenhorst, Rolls, et al., 2010). This consisted of 2 mM $NaHCO_3$ and 15 mM KCl dissolved in water. For the pilot scanning, mineral water was used as the base and the necessary ions added afterwards, which resulted in too high a hypertonic solution. This issue was then rectified and the mineral changed to distilled water for subsequent scanning sessions, with the necessary salts added.

2.4.3 – Change of design

The original design of the MRI component of the study involved the participant attending a two-hour session composed of 5 separate 20-minute runs. After resolving the issues regarding the mouthpiece and the rinse solution during the pilot testing, the first participant still ended the experiment partway through the third run, citing the length of time they had to lie down. Therefore, it was decided that the experiment would consist of six separate 15-minute runs conducted on two days no more than 10 days apart to

minimise participant dropout. The run length was also reduced to 15 minutes from 20 minutes.

2.5 – fMRI data acquisition

2.5.1 – fMRI parameters

Functional imaging data were acquired using a 3T Skyra (Siemens) scanner. Whole-brain T2*-weighted echo planar images (EPIs) were acquired with a repetition time of 3000 milliseconds, echo time of 30 milliseconds, flip angle of 90º, and 51 axial oblique slices with 3-mm isotropic resolution. A total of 300 volumes were acquired, for a total imaging time of 15 minutes and 10 seconds. A high-resolution structural MP-RAGE scan for normalization purposes was acquired beforehand (voxel size, 1×1×1 mm; repetition time, 2300 ms; echo time, 2.98 ms; inversion time, 900 ms; flip angle, 9°; total scan time, 5 min 3 s)

2.5.2 – Trial design

As many repetitions per stimulus were required to maximise the signal-to-noise ratio, the trials needed be as succinct as possible and have as many runs as possible. A trial (Fig. 2.11) tested two rating scales at a time. The trials were split into two types, namely rating and bidding. During the rating trials, the participants provided psychophysical ratings on sweetness and thickness of the stimuli, whereas during bidding trials they performed the BDM task and rated fat content on a binary scale. The two trial types were interleaved, such that in total each stimulus had 9 rating trials and 9 bidding trials randomly presented over the course of 6 runs.

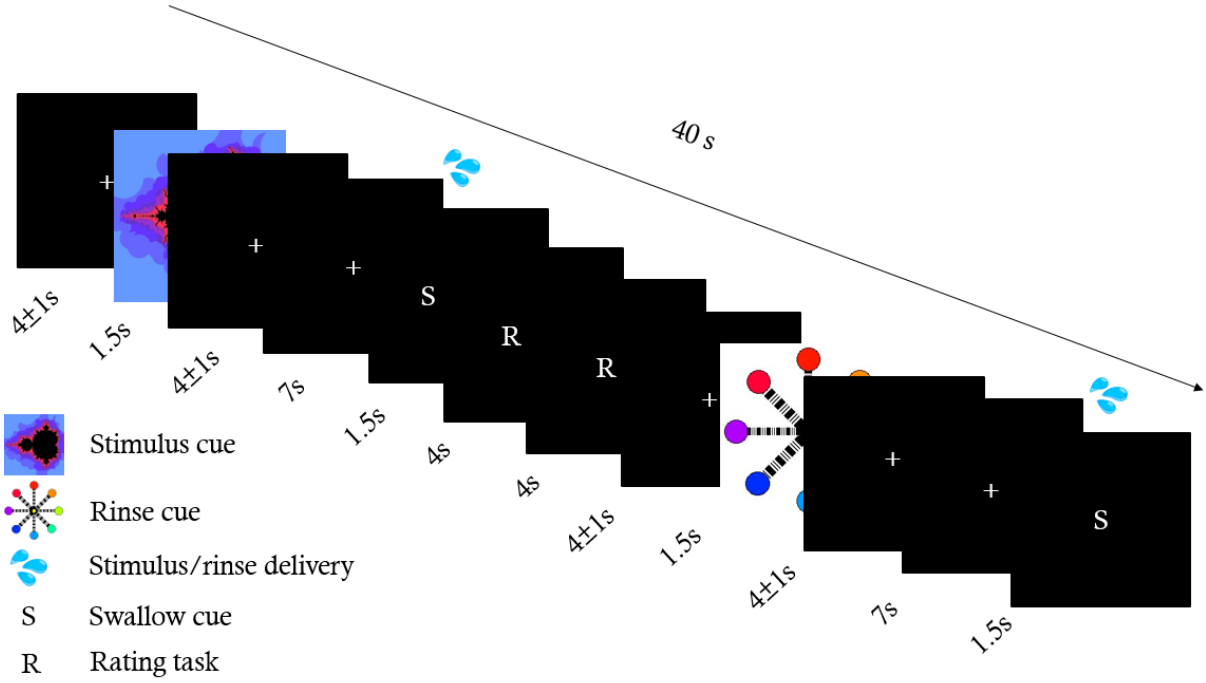

Figure 2.11. Trial design. The trial consists of an inter-trial interval (ITI) of 4s (jittered), followed by the stimulus cue, an inter-stimulus interval (ISI) of 4s (jittered), a delivery and tasting period of 7s, a swallow cue, two rating tasks each taking 4s, an ISI of 4s (jittered), a rinse cue, an ISI of 4s (jittered), a rinse delivery and tasting period of 7s and a final swallow cue before starting the trial again from the ITI. Each trial takes an average of 40s.

2.5.3 – Data pre-processing pipeline

The first 6 volumes of each run were removed to allow for scanner equilibration. Subsequently, the raw fMRI data were put through an in-house pre-processing pipeline developed using SPM12 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, London, UK) on MATLAB (MathWorks, MA, USA) as follows:

1. Slice Timing Correction
2. Day 1 Realignment and Reslicing (Runs 1, 2 and 3)
    a. 7th Degree B-spline interpolation
3. Day 2 Realignment and Reslicing (Runs 4, 5 and 6)
    a. 7th degree B-spline interpolation
4. Segmenting and Skull-stripping of structural scan
    a. SPM canonical Tissue Probability Map
    b. Grey matter, white matter and cerebrospinal fluid used
5. Coregistering of Day 1 and Day 2 data to stripped structural scan

For multivariate pattern analysis (MVPA) only steps 1-5 are used. For univariate general linear models, the subsequent steps were also conducted.

6. Normalising data to MNI space
    a. 7th Degree B-spline interpolation
    b. Resultant voxel size [2 2 2]
7. Smoothing data
    a. [6 6 6] Full Width at Half Maximum (FWHM) Gaussian kernel

Prior to running the respective analyses, global effects from the fMRI time series were removed using voxel-level linear model of the global signal (LMGS) in order to minimise effects correlated with global fluctuations of the signal (Macey, Macey, Kumar, & Harper, 2004).

2.6 – Ad-libitum eating test

This test took place in the Translational Research Facility (TRF) of the Wellcome-MRC Institute for Metabolic Sciences (IMS) in Addenbrooke's Hospital, Cambridge, with the collaboration of Prof. Sadaf Farooqi.

2.6.1 Stimuli

Using a design based on a previous ad-libitum eating test (van der Klaauw et al., 2016), we were interested in specific nutrient preferences that may be related to the neural signals recorded using fMRI. In order to examine this, we used an ad-libitum eating task where the participants were allowed to choose between three curries that were equivalent in terms of visual appearance and spicing but had varying nutrient compositions. The curries were based on the chicken korma from a previous study (van der Klaauw et al., 2016) but changed such that the protein component was from a popular meat replacement (Quorn, UK) such that vegetarian participants were not necessarily excluded from the study. With the consultation of the research staff the TRF, Prof. Farooqi and the head chef of the TRF, nutrient compositions of the curries were modulated such that they had three distinct fat and sugar levels, following a similar ratio to that of the liquid milkshakes used in the behavioural pre-testing and fMRI component of the study (Table 2.11).

Table 2.11

Nutrient and Caloric Composition of the Three Curries in the Ad-Libitum Test

	High-Fat Low-Sugar	**Medium-Fat Medium-Sugar**	**Low-Fat High-Sugar**
Energy (kcal/100g)	182	137	102
Fat (g/100g)	10	4.5	1.5
of which saturates	1	0.6	0.8
Carbohydrate (g/100g)	19	20	18
of which sugars	6.1	7.1	7.2
Fibre (g/100g)	1.7	1.7	2.1
Protein (g/100g)	3.4	3.4	3
Salt (g/100g)	0.71	0.69	0.29

2.6.2 Procedure

After their second scanning session, participants were invited to a fourth visit, in which they were told that they would perform a behavioural rating task involving solid food in addition to completing questionnaires on their eating habits. They were also informed that, as the rating of solid foods may change depending on their satiety levels, they should adhere to the breakfast options we provided (Appendix A), all of which would have around 250 kcal. They were then scheduled in for a one-hour testing session around lunchtime (between 12pm to 1pm) at the TRF.

Upon arrival, the participant was taken up to the facility and asked to leave their belongings in a locker. They were then taken to a testing room and asked to complete a pre-testing questionnaire asking about their hunger and thirst levels in addition to their adherence to the breakfast guidelines provided. Subsequently, the participant was given three small portions of the experimental curry dishes and asked to rate them on specific psychophysical scales (c.f. Appendix A). The positioning of the curry dishes was always randomised by the head chef and the researcher was blinded to the positioning until after the experiment. The participant was also provided with 100ml of water to cleanse the palate in between samples.

After the tasting test, the participant was moved to a different corner of the room to perform a computer-based Stroop Colour and Word Test (Stroop, 1935), which served as a distractor task, for two minutes. During this time, the researcher informed the TRF staff through a text message that the participant was ready for lunch. At the end of the Stroop Test, the TRF staff entered the room and asked the participant if they would like to stay for lunch and are informed that they would need to be back in half an hour to complete a final questionnaire. The participant was then taken a separate lounge and shown the three curries (in randomised positions), being informed that they should sample all of them and have as much as they wanted. The participant was informed that they would be called again in about half an hour, which was timed by the TRF staff. At the end of the 30 minutes, participants were taken to the testing room again to complete the post-test questionnaire (Appendix A) and for debriefing and their remuneration.

Chapter III – Behavioural Results and Modelling

3.1 –Introduction

Chapter II described in detail the selection process for the psychophysical ratings used in the study. This section will focus on the perceived inter-stimulus differences through the use of specific psychophysical ratings. Further to this, we will also investigate the extent to which these psychophysical ratings are related to both nutrient content and subjective value.

3.2 –Participants

For the behavioural modelling, 31 participants (10 female) took part in the experiment. Of those tested, some took part when testing the final stimulus set, whereas others participated in the behavioural pre-testing but did not continue to MRI scanning for various reasons, such as scheduling clashes. However, data from all those who completed the pre-testing component of the study were used in this chapter.

3.3 – Psychophysical Ratings

3.3.1 – Sweetness

Figure 3.1 describes the sweetness ratings of the factorial and the control stimuli across all 31 participants. Notably, a two-way analysis of variance (ANOVA) shows that the sweetness ratings are significantly higher for high-sugar stimuli than they are for low-sugar stimuli, indicating that the addition of sugar does indeed lead to a perceptible difference in sweetness [$F(1,673) = 462.89$, $p<.0001$], and the addition of fat in the factorial stimuli seems to enhance the perceived sweetness to a smaller degree [$F(1,673) = 94.76$, $p<.0001$], with a small fat × sugar interaction effect observed [$F(1,673) = 20.18$, $p<.0001$]. Interestingly, increasing fat-like textural properties in the control stimuli through CMC or soya cream does not have this effect, although a small but statistically significant difference in the than the more viscous control stimuli [$F(485)=7.63$, $p=.0005$ after Dunnet's post-hoc correction].

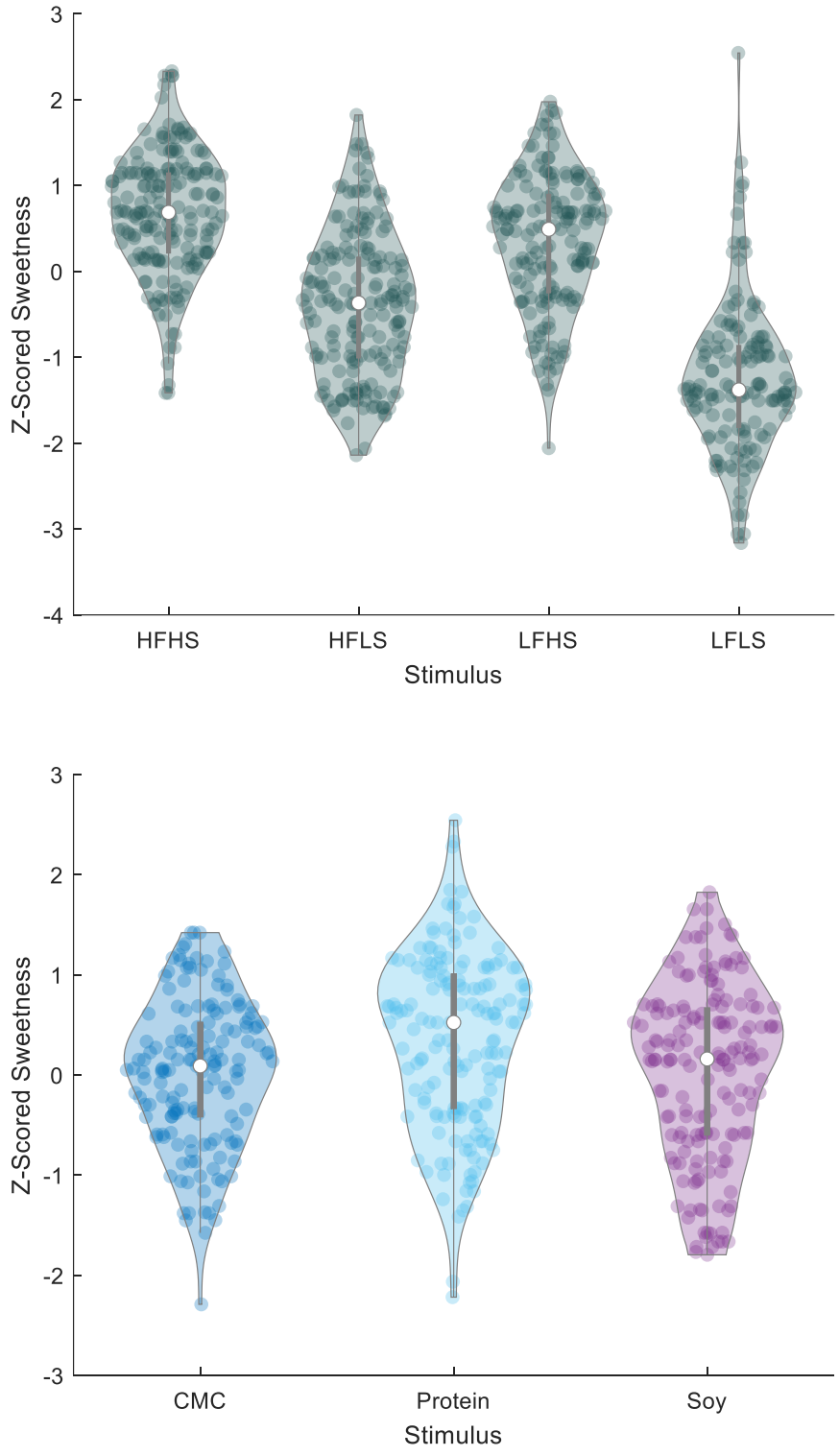

Figure 3.1. Z-Scored Sweetness Ratings.

3.3.2 – Thickness

The thickness ratings also appear to be modulated by the nutrient content of the stimuli, specifically the fat content in the factorial stimuli. As seen from Figure 3.2, high-fat stimuli are perceived to be thicker than low-fat stimuli [$F(1,673) = 824.58$, $p<.0001$], and the addition of sugar also appears to increase thickness ratings [$F(1,673) = 61.07$, $p<.0001$], with a slightly less marked interaction effect than the sweetness ratings [$F(1,673) = 6.00$, $p = .0145$]. These results indicate that adding fat to liquid food stimuli does increase the perceived thickness of oral food stimuli.

Within the control stimuli, the soya fat stimulus has the highest mean thickness rating, with the CMC stimulus being second and the high-protein stimulus have the lowest mean thickness rating. The more viscous control stimuli have a higher mean thickness rating than the protein stimulus [$t(492) = 7.33$, $p<.0001$]. This indicates that the higher viscosity elicited by the addition of soya cream or carboxymethyl cellulose is perceived by the participants through the increased thickness of oral stimuli.

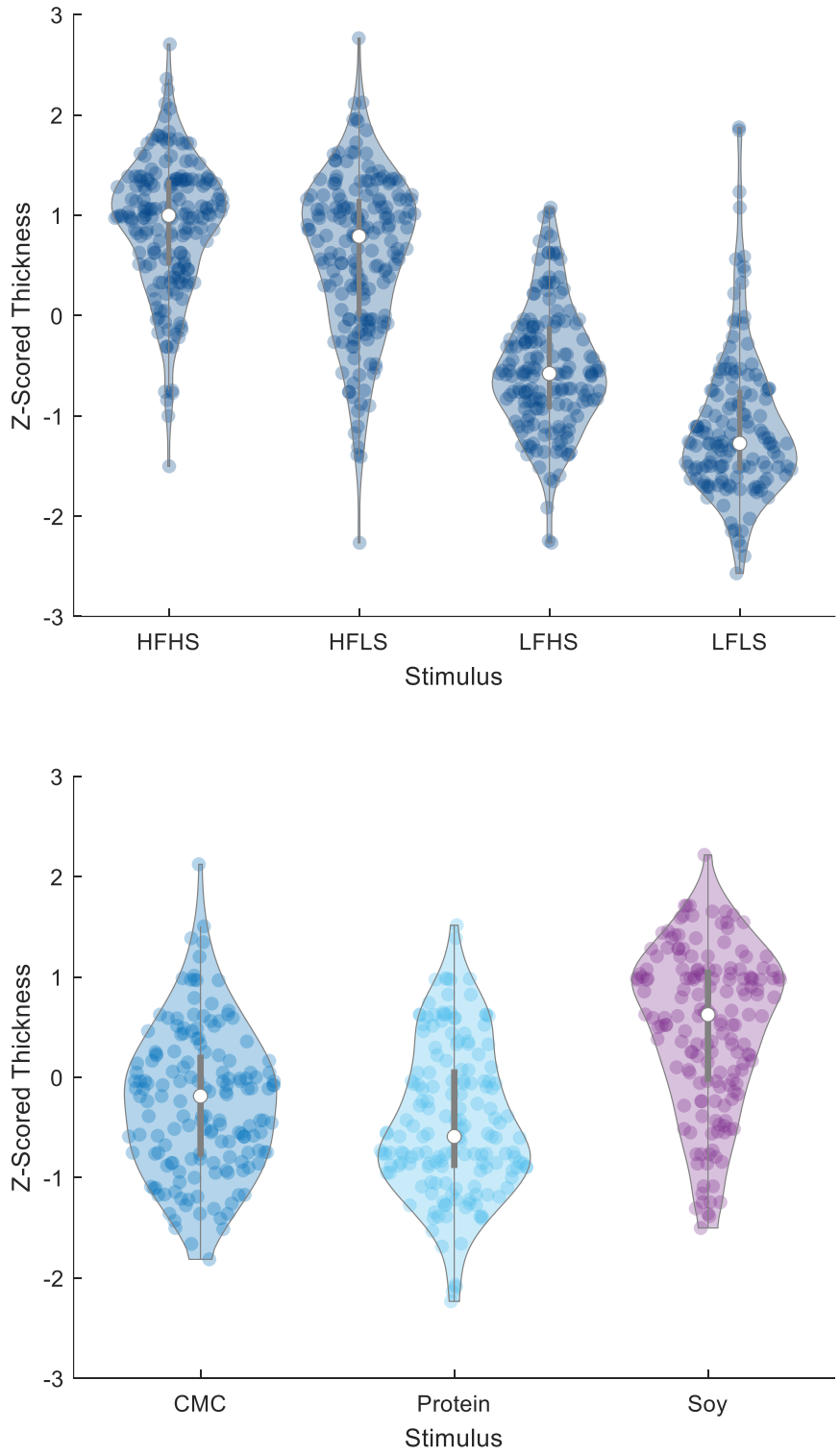

Figure 3.2. Z-Scored Thickness Ratings.

These results indicate that the addition of fat does indeed modulate oral textural properties in the form of perceived thickness. This is likely due to the increased oral viscosity, which is perceived and expressed by participants in terms of how thick they perceive the stimuli. Figure 3.3 shows that the measured shear viscosity each stimulus bar the CMC stimulus is positively correlated with its perceived thickness. Notably, the thickness scores of the CMC are lower than those of the HFHS and HFLS stimuli, which breaks the strong correlation when the control stimuli, especially the CMC stimulus, are introduced. However, it is still perceived as relatively thick in comparison to the lower-viscosity stimuli, which indicates that they work well as controls for dairy cream, although the slightly lower thickness ratings for the CMC may indicate that participants' perceptions of the thickness of oral stimuli may not be solely modulated by viscosity.

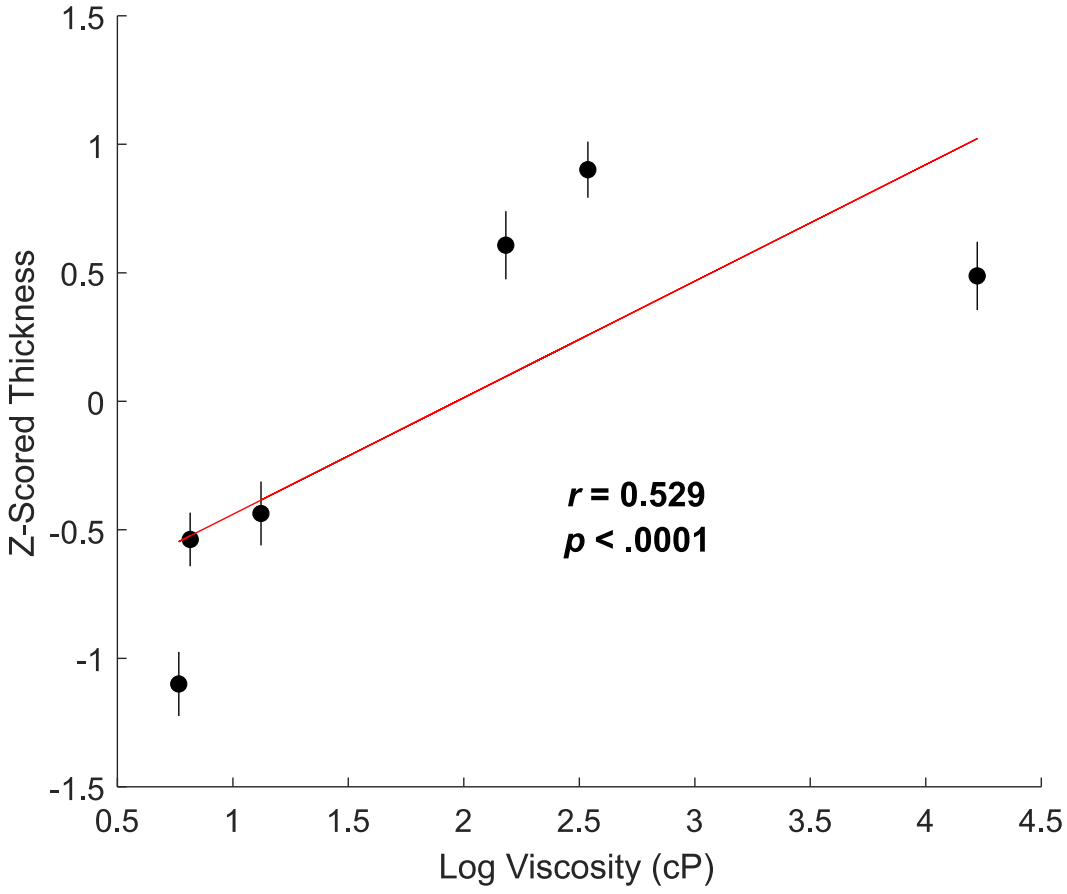

Figure 3.3. Plot of Z-Scored Thickness Ratings against the Log of Viscosity of the Factorial Stimuli.

3.3.3. – Oiliness

Oiliness ratings provided by participants also reflect the fat contents of oral food stimuli. A two-way ANOVA of nutrient content and oiliness ratings shows that, in the factorial stimuli, oiliness ratings are primarily modulated by fat content [$F(1,673) = 197.26$, $p<.0001$] and, interestingly, sugar content also mildly affects oiliness ratings [$F(1,673) = 35.26$, $p<.0001$], with a small interaction effect between fat and sugar being observed [$F(1,673) = 6.02$, $p = .0144$]. Notably, in the control stimuli, the soya cream stimulus also has a high oiliness rating, whereas the CMC stimulus is at about the same level as the protein stimulus. This indicates that the mere addition of CMC is not sufficient to create the mouthfeel related to the sensation of oral fat. This may be due to an innate nature of CMC, such as its shear-thinning properties or sensitivity to temperature, that may affect the subjective perception of oiliness. Interestingly, while we see a strong correlation between the normalised coefficient of sliding friction of all experimental stimuli, removing the CMC stimulus from the correlation analysis improves this correlation even further (Fig. 3.5; $r = -0.401$, $p < .0001$) thereby indicating that the CMC stimulus does not elicit the same oiliness ratings associated with the decrease in CSF.

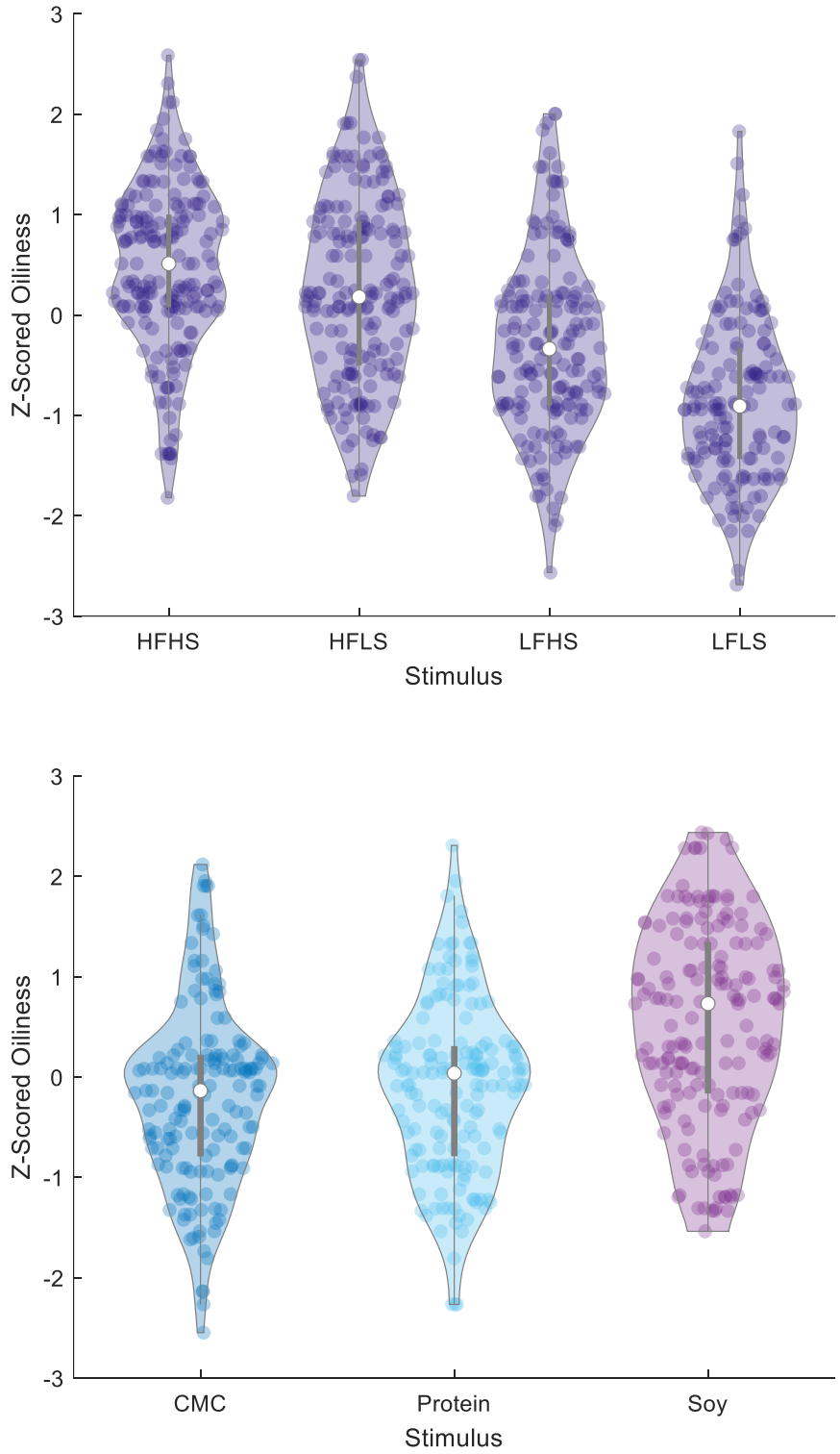

Figure 3.4. Z-Scored Oiliness Ratings.

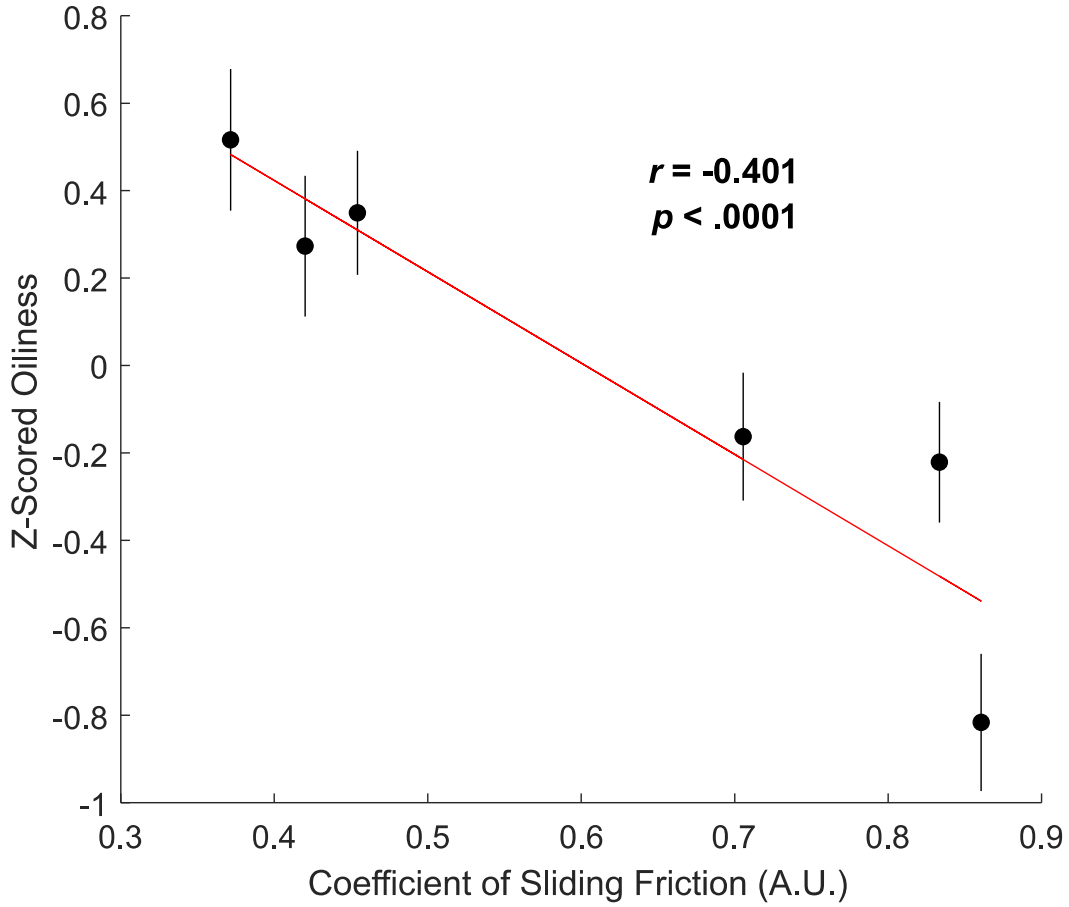

Figure 3.5. Correlation of Z-Scored Oiliness with Coefficient of Sliding Friction.

3.4 – Subjective Value

Each participant's subjective value for each stimulus was measured using a modified version of the Becker-DeGroot-Marschak auction (Becker et al., 1964) which, as explained in Chapter II, is an incentive-compatible measure of subjective value. This value is expressed in their willingness to pay (WTP) during each trial, which is then compared to a computer bid. A higher WTP indicates that they are willing to sacrifice more of their budget to consume the stimulus they have just tasted.

3.4.1 – Descriptive

Across all participants, the HFHS stimulus had the highest mean WTP rating, with LFLS having the lowest WTP and the HFLS and LFHS stimuli in between (Figure 3.6). Meanwhile, the control stimuli did not seem to have as high a mean WTP as the HFHS, indicating that the various controls added were not able to fully replace the reward value of fat. The control stimuli were specifically chosen for their properties, that is, the

potential ability to mimic the textural property of fatty cream, the use of an alternative macronutrient instead of fat or the use of a plant-derived cream replacement. Therefore, these controls would be good starting points to model the determinants of reward value.

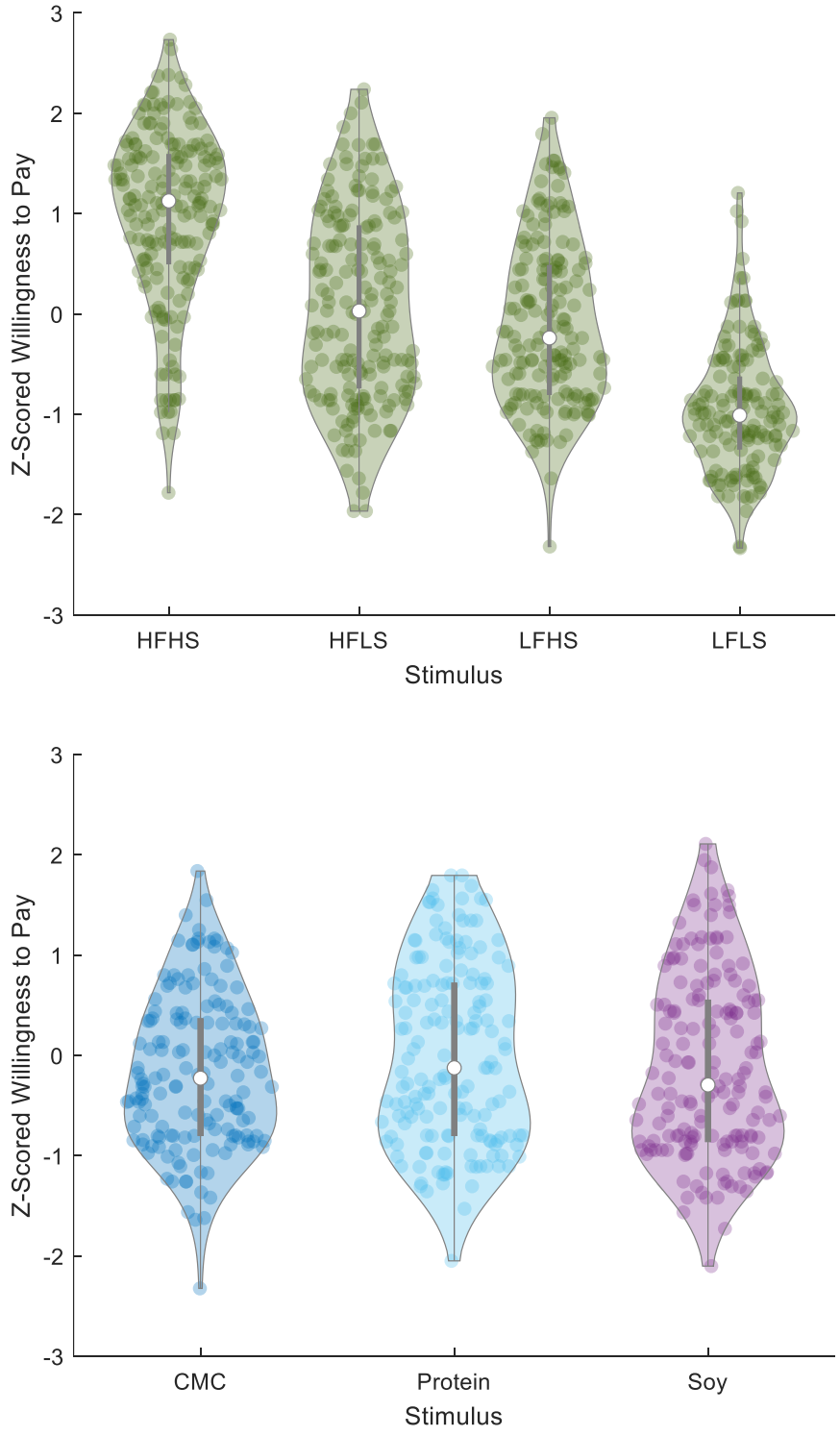

Figure 3.6. Z-Scored Willingness to Pay for all Stimuli**.**

While the general trend applies across the span of all participants, WTP values varied greatly among participants, confirming that values were subjective and not trivially determined by objective stimulus properties. Figure 3.7 shows three individual participants' WTP ratings over the course of the experiment, the stark differences among which indicates that participants value different macronutrients in varying manners, with some participants assigning greater value to high-fat stimuli than they do to high-sugar stimuli. Therefore, attempts at investigating the determinants of individual subjective value should focus on modelling subjective value within individuals before comparing these models across all participants. Using a two-level analysis such as this allows individual differences in the various factors that may influence WTP to be tailored specifically to each participant.

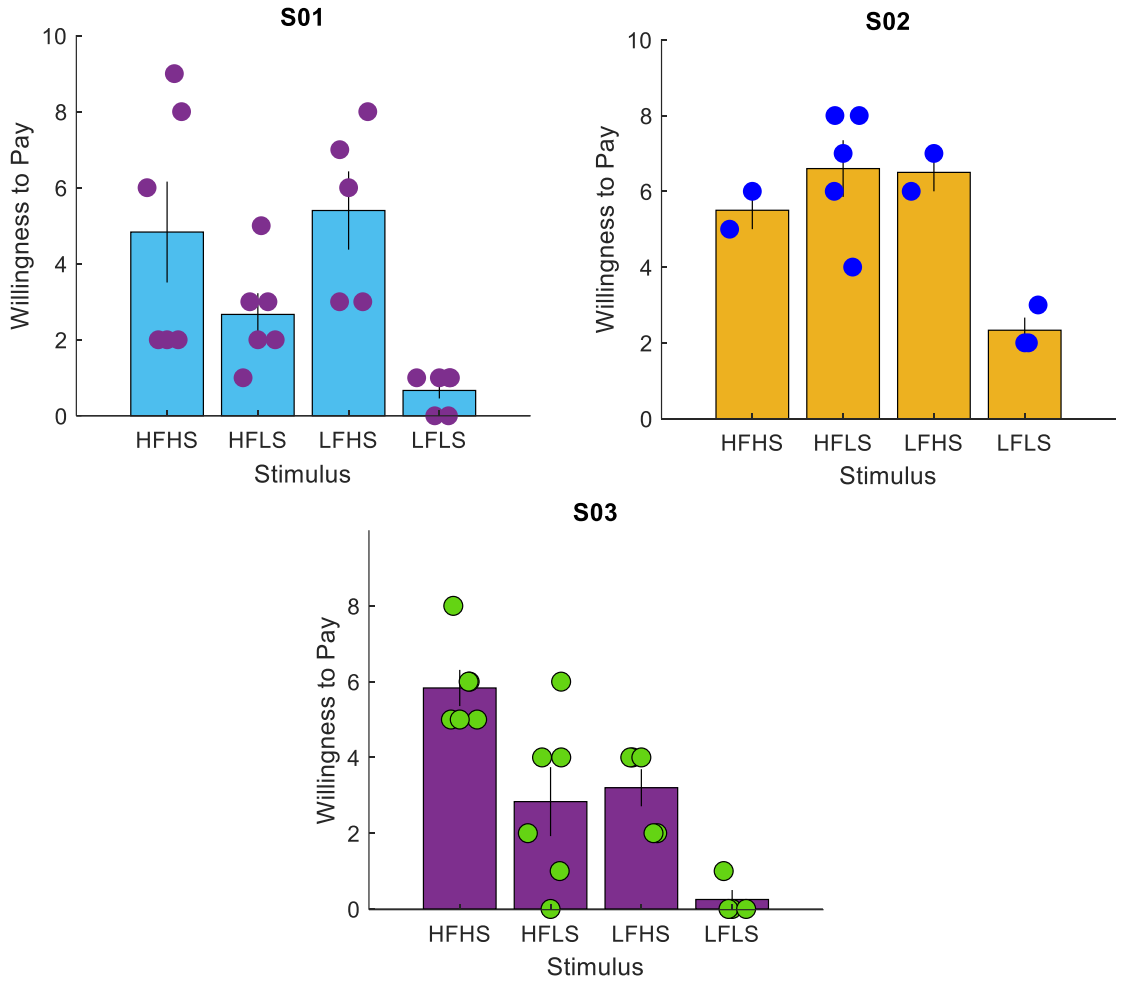

Figure 3.7. Individual BDM values for Factorial Stimuli.

3.4.2 – Determinants of Subjective Value

Having established that determinants of subjective value need to be modelled for each participant, we now go on to explore the various models that can be used to explain what best determines individual subjective value.

3.4.2.1 – Caloric Load

One of the most fundamental questions we first asked was the extent to which the caloric load of each stimulus affected the subjective value. Specifically, the extent to which the caloric load of each stimulus contributed to variation in the subjective ratings were of interest. On a fundamental biological level, foods with higher caloric loads are advantageous for the organism, as they would maximise the benefit of ingestion and reduce the effort required in procuring food. Furthermore, post-ingestive effects have been shown to gradually modulate responses to non-gustatory cues, such as visual cues,

to prefer foods that have higher caloric loads while still tasting the same as their low-calorie counterparts (de Araujo et al., 2013). Therefore, it stands to reason that caloric load would play a crucial role in the determination of the subjective value of a food.

In order to examine this, a general linear model (GLM) was calculated for each participant, where WTP is a dependent variable and the caloric load of the stimulus the independent variable as such:

$$WTP = \beta 1 \times Caloric\ Load + c$$

The betas for each participant indicate the extent to which the variable of interest, in this case caloric load, influences that participant's subjective value of the stimulus tasted. Figure 3.8 shows the distribution of the standardised betas of each participant for caloric load, a large number of which are above 0. The lack of conformity of the data to a normal distribution indicates that a non-parametric test such as the Wilcoxon signed rank test is required. Indeed, a Wilcoxon signed rank test indicated that these betas were significantly higher than zero, Z = 4.66, p<.0001, thereby supporting the notion that caloric load is a contributing factor to subjective value.

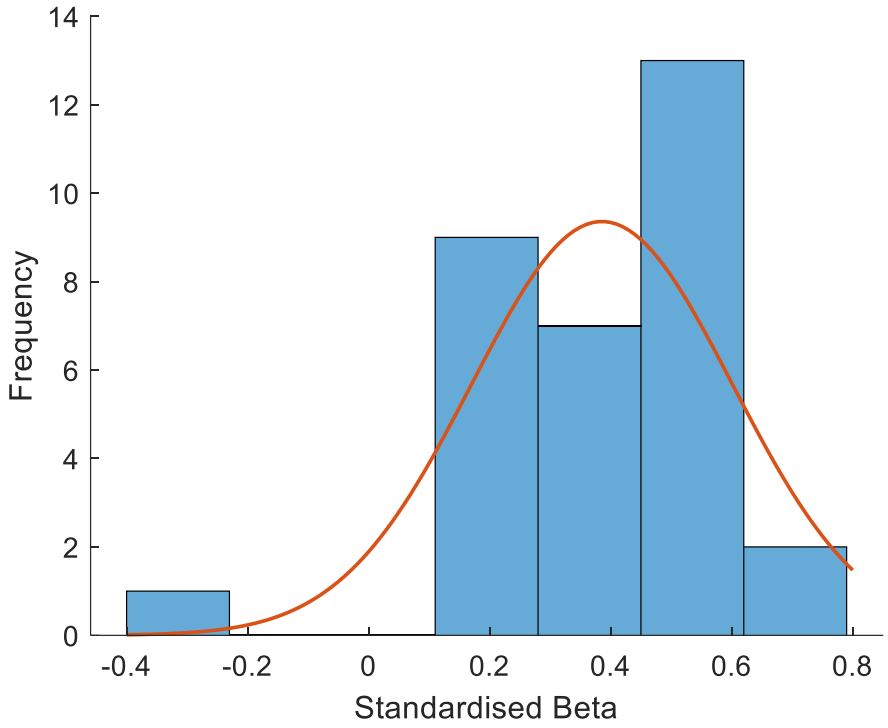

Figure 3.8. Histogram of Betas in Caloric Load GLMs. GLMs were conducted within each participant with WTP as the dependent variable and caloric load as the independent variable. The betas for caloric load are shown here (mean = 0.386, S.D = 0.216) in addition to a normal distribution, showing that the data required a non-parametric test of significance.

3.4.2.2 – Macronutrient Content

However, although these data show that calorie content does indeed contribute to the reward value of foods, the role of the macronutrient composition of the food ingested also needs to be explored. The existence of behaviours such as fat-specific hyperphagy (van der Klaauw et al., 2016) and protein leveraging (Martinez-Cordero et al., 2012; Simpson & Raubenheimer, 2005) suggest that there are specific mechanisms that both detect the presence of these specific nutrients in different foods and factor them into the overall reward value of said food items. Hence, if nutrient-specific orosensory mechanisms were able to distinguish specific macronutrients and then compute these into a reward value, we should see that the specific macronutrient composition provides a good predictor for subjective value.

In order to test this possibility, GLMs of WTP were run with the main macronutrients as regressors. One model used fat, sugar and protein content as regressors, whereas another used only fat and sugar, as such:

$$WTP = \beta1 \times Fat + \beta2 \times Sugar + \beta3 \times Protein + c, \text{ or}$$

$$WTP = \beta1 \times Fat + \beta2 \times Sugar + c$$

As seen from Figure 3.9, both fat and sugar content seem to contribute substantially to subjective value [$t(30) = 7.91$, $p<.0001$ and $t(30) = 5.39$, $p<.0001$ respectively]. Protein content, however, has a weaker but still significant effect on the variance in WTP [$t(30) = 2.55$, $p = .0157$]. Moreover, models were stronger when only fat and sugar content were used as regressors [Fig. 3.9; $t(30) = 7.62$, $p<.0001$ and $t(30) = 5.59$, $p<.0001$ respectively]. An analysis of the Akaike Information Criteria (AIC), a measure of the trade-off between the goodness-of-fit of models and the risk of overfitting (Akaike, 1974), indicates that the second model with only fat and sugar is a better model of subjective value through the statistically smaller AIC [$t(30) = -2.80$, $p = .0088$]. Crucially, the small contribution of protein content to WTP may be due to the fact that only one specific stimulus had a high protein content, whereas the others had similar protein content. Meanwhile, fat content and sugar content are both modulated extensively within the stimulus set used. Therefore, the data indicate that a fat and sugar content contribute more to subjective value within the current stimulus set.

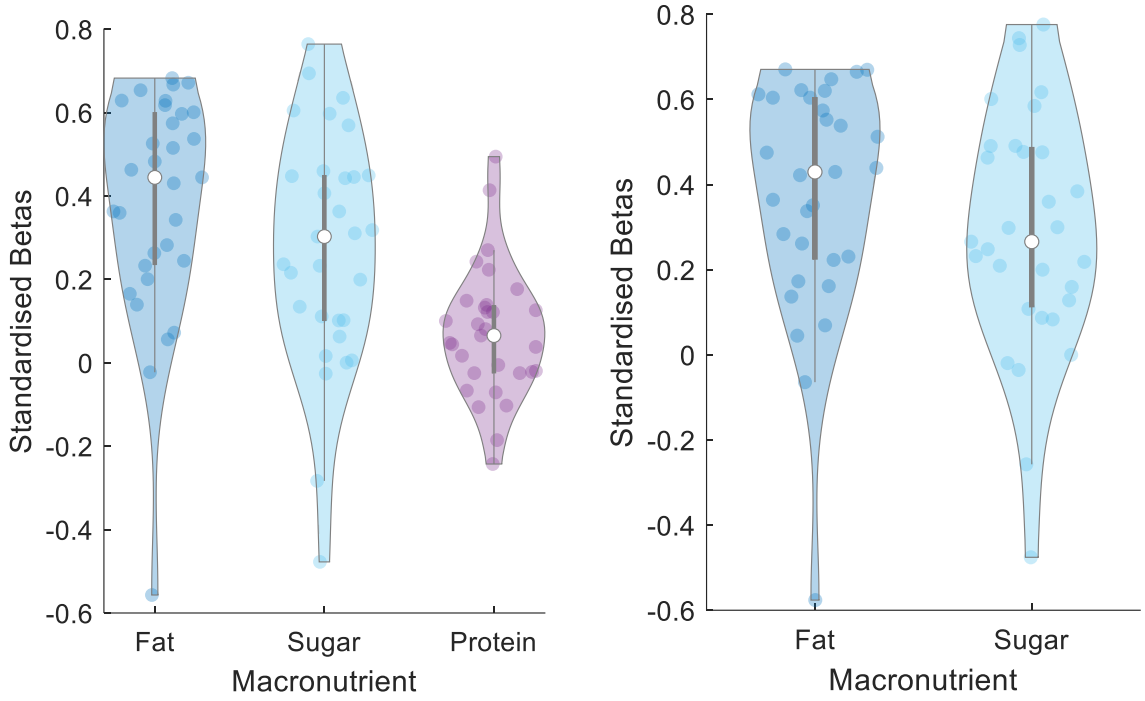

Figure 3.9. *Left* Plot of Standardised Betas of GLMs using Fat, Sugar and Protein Contents. *Right* Plot of Standardised Betas of GLMs using only Fat and Sugar Contents. Protein content does not contribute extensively to the model, as can be noted from the low betas and the lower AIC of the second model.

3.4.2.3 – Textural Parameters

Establishing the link between the macronutrient composition and subjective value begs the question of the specific mechanisms by which these nutrients are sensed. Specifically, mechanisms of fat detection are still widely disputed in the literature (B. V. Kulkarni & Mattes, 2014; E. T. Rolls et al., 2018; Tucker et al., 2014), which is an aspect that the various high-fat stimuli in the stimulus set can help explore. Through rheological and tribological measurements of the stimuli, the viscosity and the coefficient of sliding friction of the stimuli were obtained. These measurements were then entered as regressors, alongside sugar concentration, to explore the effect of these specific textural parameters on subjective value.

Figure 3.10 shows the distribution of the betas of sugar concentration, viscosity and the coefficient of sliding friction. As expected, sugar concentration has a relatively high beta above zero [mean = 0.380; $t(30) = 8.18$, $p<.0001$], and the coefficient sliding friction has a relatively low beta well below zero [mean = -1.05; ; $t(30) = -10.4$, $p<.0001$]. Interestingly, viscosity is also below zero and close to that of CSF [mean = -0.959; $t(30) = -9.25$, $p<.0001$], although the fact that stimuli high in fat are also high in viscosity, such

that a positive relationship would have been expected. However, the negative relationship may be due to the strong negative correlation in the stimulus set between viscosity and CSF, such that they do not explain two separate variables. Furthermore, the only time this negative correlation is broken is in the CMC stimulus, wherein the high increase in viscosity is not compensated by as high a reduction in CSF. Notably, the CMC stimulus had a relatively low mean WTP, indicating that it was not particularly popular. Thus, it seems likely that the negative relationship between WTP and CSF captured the positive valuation of high-fat stimuli, whereas the negative relationship between WTP and viscosity may account for the relatively lower value of the (high-viscosity) CMC stimulus. This could have been the reason for the negative relationship between viscosity and subjective value in this model.

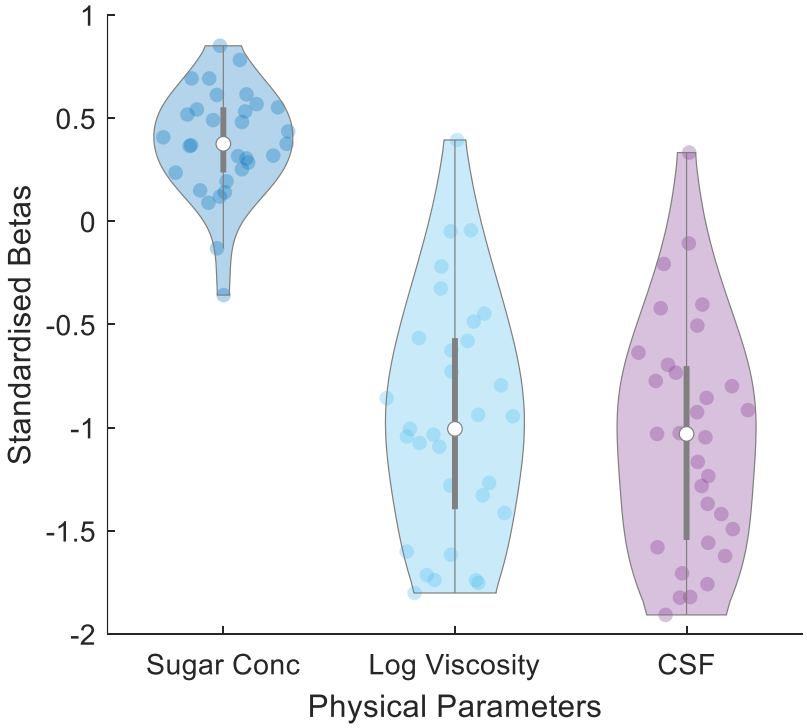

Figure 3.10. Plot of Standardised Betas of GLMs using Sugar Concentration, Log Values of Viscosity and Normalised Coefficient of Sliding Friction.

3.4.2.4 – Subjective Ratings

Having established that the physical parameters of sugar concentration, viscosity and the coefficient of sliding friction are determinants of subjective value, it is important to establish which of the tested psychophysical ratings contribute to subjective value. As these specific psychophysical ratings reflect how participants report their subjective perceptions of each stimulus, these ratings should account for individual differences, such

as differences in the ability to sense sugar levels or different palatal and tongue formations that may result in differing actual sliding friction properties of the oral food stimuli.

This possibility was initially tested using all psychophysical ratings apart from the perceived protein content, as the participants' ratings of protein content did not match that of actual protein content (Fig. 3.11), indicating that participants are generally unable to judge protein content through taste, or that they do not explicitly associate the sensory differences between low-protein and high-protein stimuli as being due to protein content. Hence, the main psychophysical ratings used initially were sweetness, thickness, oiliness and perceived fat content.

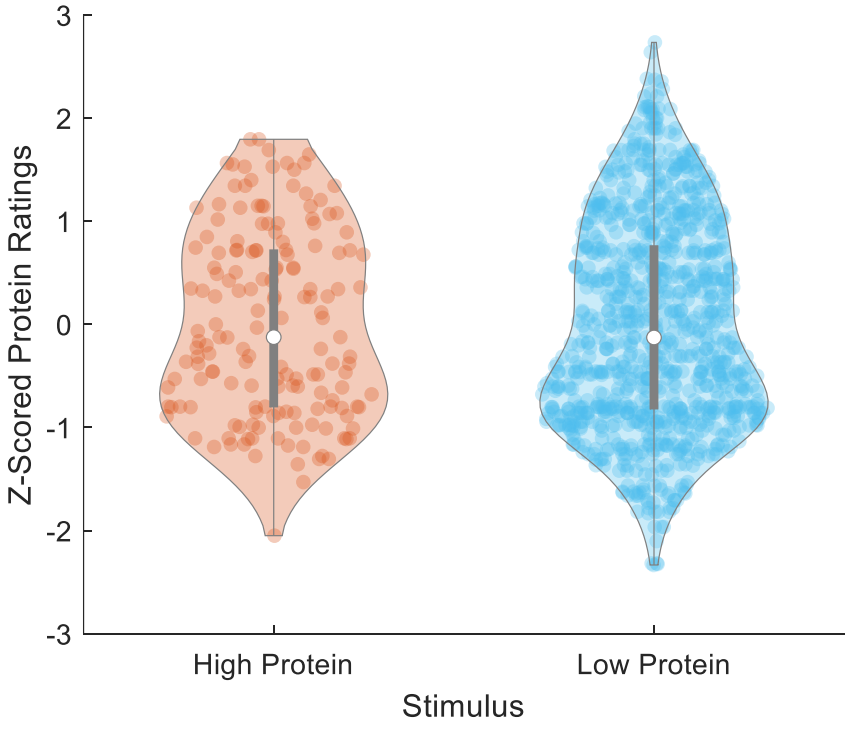

Figure 3.11. Z-Scored Normalised Ratings of Protein Content of High Protein and Low Protein Stimuli.

Figure 3.12 (*Top*) shows the distribution of betas of the four ratings. Notably, sweetness and thickness had high mean betas (mean = 0.295, $t(30) = 7.09$, $p<.0001$ and mean = 0.410, $t(30) = 6.86$, $p<.0001$ respectively), whereas oiliness and perceived fat content had less strong, but still significant, effects (mean = -0.164, $t(30) = -3.74$, $p = .000766$ and mean = 0.130, $t(30) = 3.57$, $p = .0012$ respectively). Therefore, another model was tested with only sweetness and thickness as regressors (Fig. 3.12, *Bottom*), where both

sweetness and thickness were good predictors of subjective value (mean = 0.366, $t(30)$ = 7.26, $p<.0001$ and mean = 0.382, $t(30)$ = 6.64, $p<.0001$ respectively). Crucially, the mean AIC for the second GLM with only two regressors was significantly lower than that of the first GLM [$t(30)$ = -3.58, p = .0012], which indicates that the second GLM provided better prediction without the risk of overfitting the data.

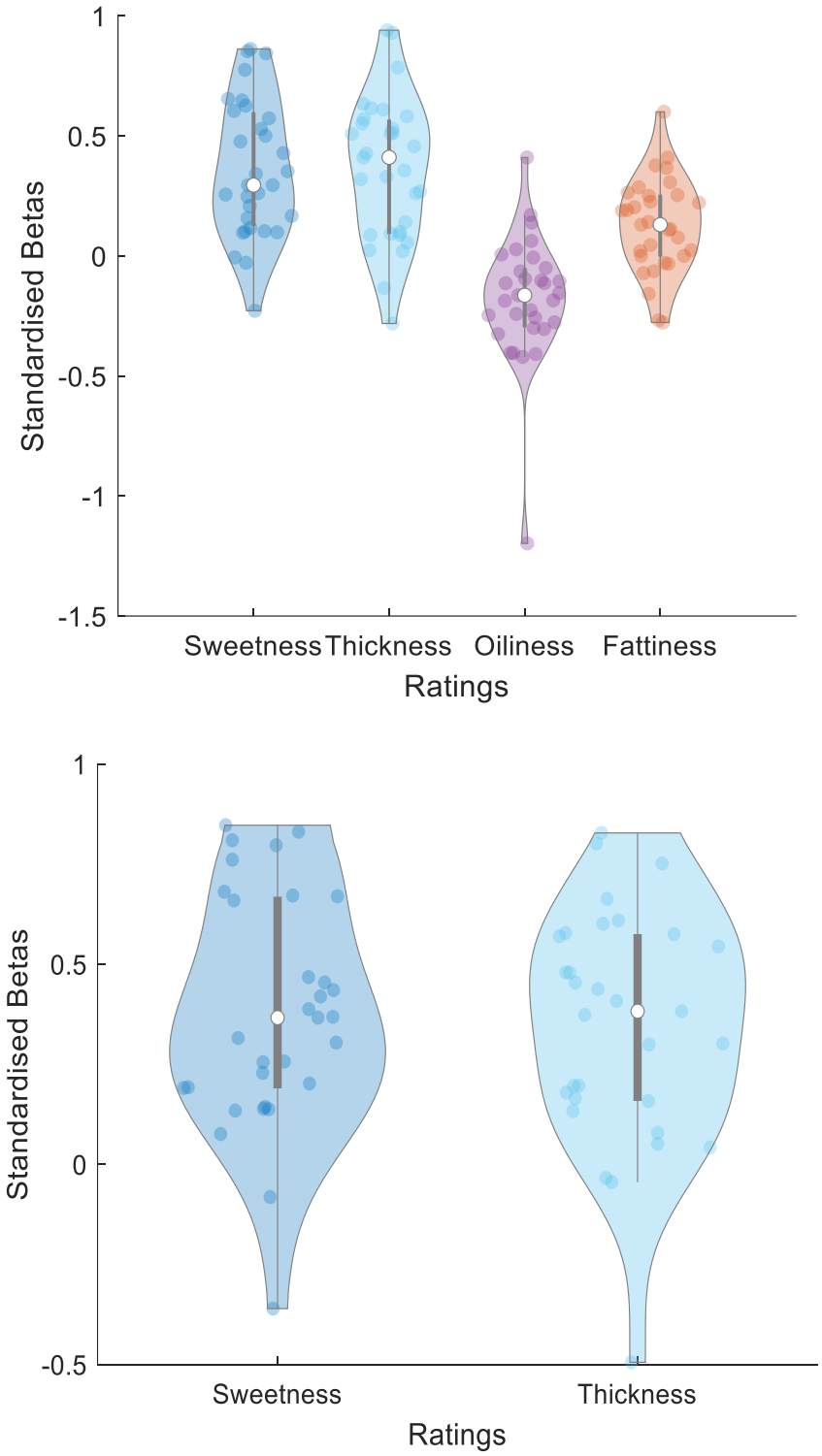

Figure 3.12. *Top* Standardised Betas of GLM using all four psychophysical ratings. *Bottom* Standardised Betas of GLM using only Sweetness and Thickness. Oiliness and perceived fat content do not contribute greatly to subjective value, such that their removal results in a more comprehensive model.

3.4.2.5 – Model Comparison

After establishing that all the previously mentioned models are able to identify determinants of subjective value, it is important to compare these models against each other to establish how subjective value is actually derived. The AIC, mentioned previously as a method of model comparison, is a measure of how much information is lost in a model. The AIC also takes into account the number of predictor variables within the model, thereby essentially showing a trade-off between how well the model fits and its parsimony. Therefore, it rewards well-fitting models and penalises over-fitting of the data, thereby being a suitable candidate for model comparison.

By generating GLMs within each participant, the AIC is unique for each model and each participant. As a result, model comparison is performed by calculating the means of the AICs of each model across all participants. These means would therefore represent how well the model fits within the population of participants. Therefore, they would show the most apt measures for predicting subjective value through the participants' WTP. Table 3.1 shows the mean and standard deviations of the AICs of the various models tested. AIC analysis indicates that the GLM using the subjective ratings of sweetness and thickness is the best model, due to the significantly lower AIC from all the other models (p<.0001 for all pairwise comparisons, which satisfies the threshold for significance after a Bonferroni correction). Interestingly, the caloric load model performed the worst, whereas the model with only sugar and fat did almost as well as the model using sugar the two textural parameters of fat. This may be again due to a lack of low-fat high-viscosity stimuli apart from the high-viscosity CMC stimulus. However, it is important to note that the sugar and fat model is essentially a 'descriptive' model, describing the actual nutrient content, whereas the model including sugar and the texture parameters is a 'mechanistic' model, in the sense that it specifies two textural mechanisms by which fat content could be sensed (with sugar being sensed rather directly from sweet taste).

Table 3.1.

Akaike Information Criteria for Each Behavioural Model

Regressors	**Caloric Load**	**Sugar Fat**	**Sugar Log Viscosity CSF**	**Sweetness Thickness**
Mean AIC (S.D.)	-143.2 (23.1)	-146.3 (24.9)	-146.4 (24.5)	-152.0 (25.6)

Notably, while the AIC offers a method of comparing the goodness-of-fit and simplicity of existing models, it does not provide an objective measure of how well a model fits in and of itself, such that if all models chosen do not fit well it would not be reflected in the AIC. However, the subjective rating GLM does seem to have a reasonably good fit, as the median *p*-values for the sweetness and the thickness regressors are .0051 and .0106 respectively. Furthermore, a visual analysis of the regressors in randomly chosen participants (Fig. 3.13) show that the residuals seem randomly distributed, confirming the models' adquate fit. Thus, the subjective ratings, specifically sweetness and thickness ratings, are the best predictors for WTP and therefore form the most important subjective components of reward value in oral food stimuli within the stimulus set tested. In addition, the texture parameters are of particular interest as they provide a mechanistic link between objective nutrient content and subjective perceptions.

In sum, these findings indicate that subjective value is derived from various components. While caloric load does indeed contribute to subjective value to a degree, subjective ratings seem to be more finely tuned to the specific macronutrient compositions of the stimuli presented. Therefore, in order to sense the nutrient composition, the participants rely on the physical taste and textural parameters – in this case, oral changes in sugar concentration, viscosity and CSF – which are then integrated into a reward value. However, subjective psychophysical ratings, a proxy for the sensations perceived by the participants during the consumption of the stimuli, are much better direct predictors for subjective value. There are various reasons that this could happen, both within and between participants. One such inter-participant reason would be that the taste receptor density on the tongue differs between participants, whereas an intra-participant reason would be the difference in saliva levels in between trials, adherence to the prescribed tongue movement or the attention the participant was paying to the stimulus delivered during that particular trial, thereby essentially accounting for all these variables when using the trial-by-trial subjective ratings. This sequence provides both an insight into nutrient components of reward value and a starting point for investigations into the neural mechanisms thereof (discussed in Chapter IV).

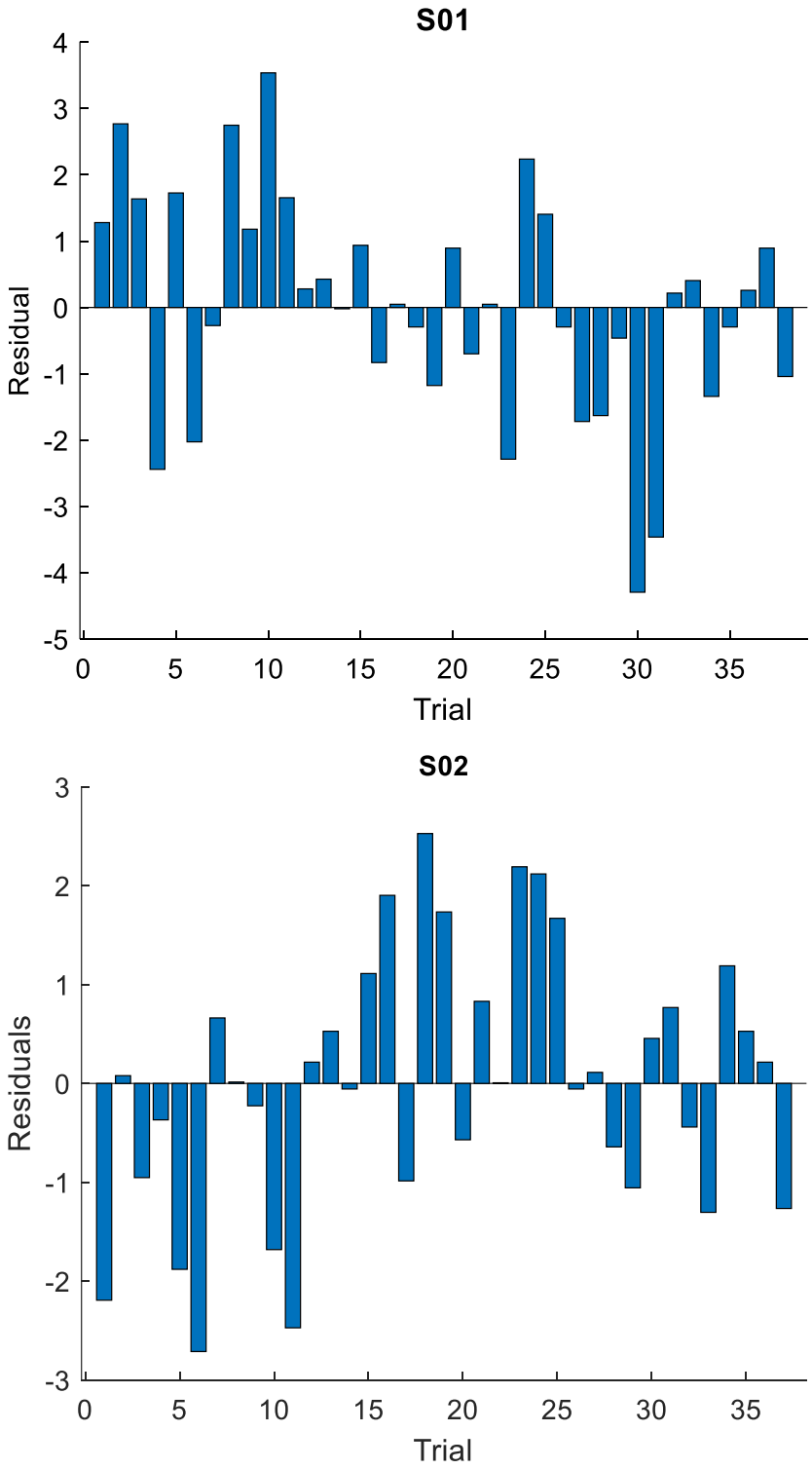

Figure 3.13. Plot of residuals of two participants (S01 and S02) against trial number. The apparently random distribution of the residuals for the GLM with only subjective sweetness and thickness as regressors

3.5 – Fat Detection

The use of the binary fat choice scale, where participants were asked to rate if the stimulus they tasted contained fat on a yes/no scale, allowed the investigation of how fat

perception differs among individuals. This is especially useful as the stimulus set had large variations in fat content as well as control stimuli in the form of protein, soya cream and CMC. The binary fat choice outcome is therefore an estimate of both the participants' perceived fat content of the stimulus and their specificity of fat sensing, that is, if they are able to sense that something is not fatty but has additional nutrients or textural properties similar to fat. This is calculated by averaging the binary choices for each stimulus over the course of the experiment, as the participant repeats the same stimulus 6 times in a pseudo-randomised order (see Chapter II for the methodology). This would then yield the fat choice probability, that is, the probability that the participant reports that particular stimulus as containing fat.

Participants reliably marked both high-fat stimuli (HFHS and HFLS) as containing fat, as most of the fat choice probability is centred around 1 (Fig. 3.14, *Top*). This shows that the participants were indeed able to sense fat in these stimuli and report its presence as expected. However, the distribution of fat choice probability in the control stimuli appears much more interesting, as there appears to be a bimodal distribution for the protein and the CMC stimuli, where participants tended to have either a very low or very high fat choice probability (Fig. 3.14, *Bottom*), indicating that fat sensitivity varies among individuals and that some individuals are more capable of distinguishing actual fat content from textural and nutrient-based changes in oral stimuli.

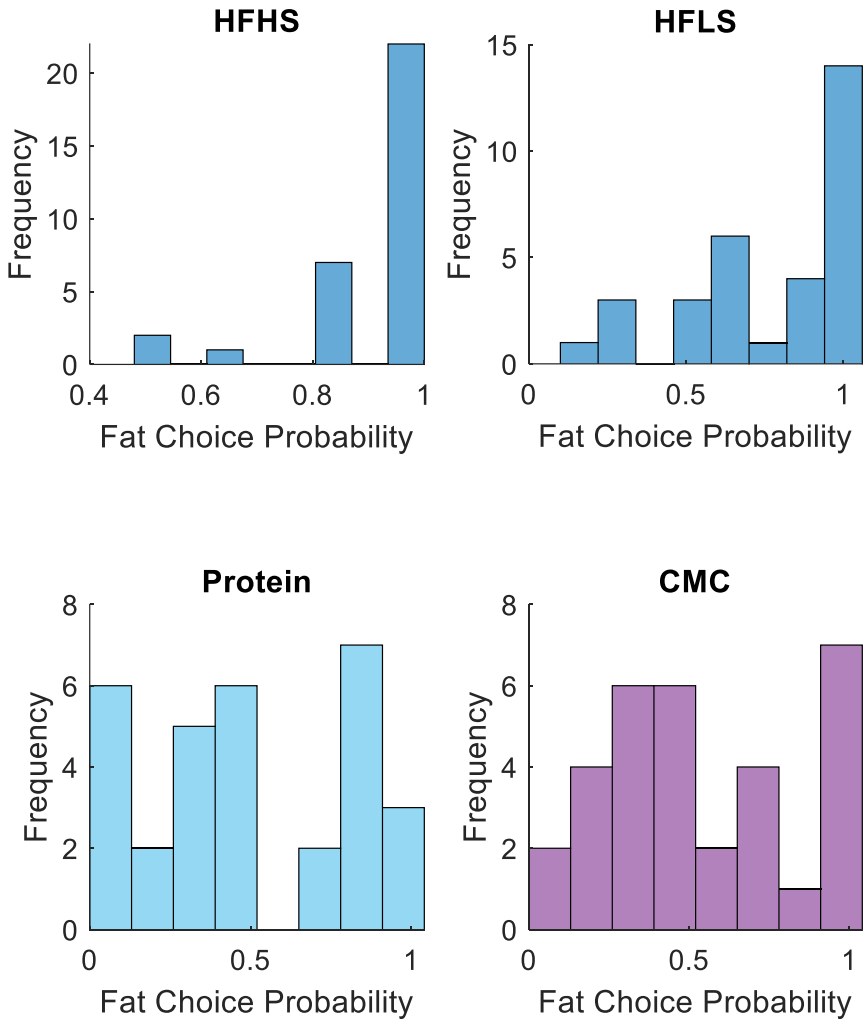

Figure 3.14. *Top* Fat Choice Probability Distribution of High-Fat Stimuli. *Bottom* Fat Choice Probability Distribution of Protein and CMC Stimuli.

The impact of the variability of susceptibility to the replacement of fat through textural or other nutrient properties is profound. One of its implications is that fat sensing differs in people, in that there may be a proportion of the population that is able to sense fat through something other than its textural properties, as they are able to report that the high-viscosity and low-CSF CMC stimulus is devoid of fat, whereas there may be a population that relies exclusively on the increased viscosity and reduced CSF to determine fat content. However, this does not negate the notion that humans sense fat through textural properties, as the increase in viscosity in the CMC stimulus is substantially higher than the reduction in CSF it elicits. Therefore, it could be that the participants who report the CMC stimulus as being fat free have finely tuned textural

sensing, such that the discordant increase in viscosity and decrease in CSF is unlike that typically found in a fatty food, whereas if the accompanying reduction in CSF were greater these participants may report the stimuli as fatty. This difference in tuning of fat detection and the mechanisms behind this, as well as the impact this has in real life needs further investigation.

Moreover, the increased fat perception in the protein stimulus as well as the CMC stimulus has vast implications in the field of nutrition and food engineering. As fat contains over twice the amount of calories per gram of protein, and CMC is an indigestible polysaccharide that does not lead to net caloric increase, they provide viable and lower-calorie alternatives to fat in foods to mimic the texture and add an alternative macronutrient. Indeed, they are already often used in low-fat foods such as yogurts in order to improve the texture and taste of the foods. However, these results indicate that there may be a substantial population that is not susceptible to this switch, such that these individuals are able to detect that the food does not contain any fat, which may in turn affect their reward values for these foods such that they do not replace higher-fat and higher-calorie foods with these lower-fat foods. Once again, this is an avenue for further research as improving the texture of the stimuli such that participants report non-fatty stimuli as fatty may help these low-fat foods to be developed such that they have more fat-like textural properties with a lower calorie density.

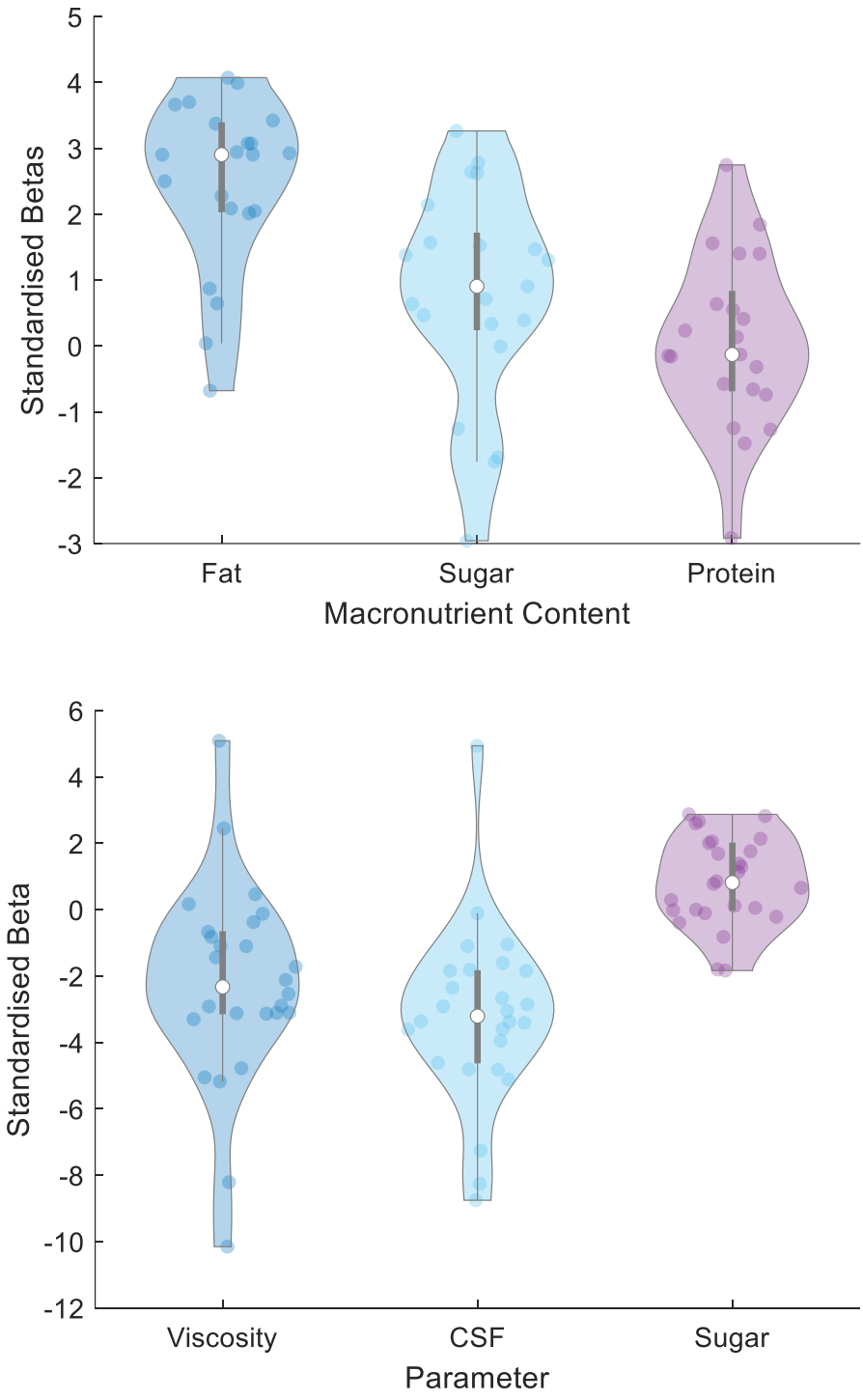

Figure 3.15. *Top* Standardised Betas of logistic regression using macronutrient components. *Bottom* Standardised Betas of logistic regression using textural and taste parameters.

However, when logistic regression analyses were performed on the fat choice outcome (Fig. 3.15, *Top*) using all macronutrient contents, protein content did not significantly predict the likelihood of the participant marking the stimulus as fatty [Wilcoxon Signed Rank test, Z = -0.0541, p = .957], whereas fat content and sugar content

were positively linked to the likelihood of the stimulus being marked as fatty [Z = 4.01, p<.0001 and Z = 3.12, p = .0018 respectively]. It is likely that the fat-replacement sensation of protein was low in comparison to actual fat and sugar, especially as the high-protein stimulus was also high in sugar with no protein variability in the other stimuli. When the textural and taste parameters were used (Fig. 3.15 *Bottom*) the viscosity and CSF of the stimuli were both predictive of the stimulus being marked as not fat-containing [Wilcoxon Signed Rank test, Z = -2.87, p = .0042 and Z = -3.54, p < .0001 respectively], whereas sugar concentration was not a predictor [Z = 1.65 p = .0981]. This result is likely due to the split between participants in their responses to the CMC stimulus, as a population of the participants would mark it as not fatty despite its high viscosity. Interestingly, AIC analysis only marks the macronutrient-based model as marginally better performing than the model with the textural parameters and sugar content. This result indicates that, in our stimulus set, the control stimuli were not able to faithfully replicate the sensation of fat, although there are trends towards potential future textural and macronutrient combinations.

3.6 – Realistic Eating Behaviour

The final component of the project involved an ad-libitum eating test in collaboration with Prof. Sadaf Farooqi of the MRC-Wellcome Institute of Metabolic Sciences (IMS) in Cambridge. This test was conducted in the Translational Research Facility on the University of Cambridge Biomedical Campus. Participants were invited to attend a taste-test session at lunchtime during which time they are asked to rate the taste of a small amount of solid food items and fill out questionnaires (see Chapter II), after which they are asked to stay for lunch. During the lunch, they are asked to eat from a buffet of three curries, all of which have well-controlled macronutrient properties. Of note, the fat content of the curries are vastly different, allowing an insight into their individual fat preference.

As the visit was conducted after the participants had completed the behavioural pre-testing and the two scanning sessions, the final component of the experiment was the participants' fourth visit. Of the 22 participants scanned, 1 did not return for the final component and 3 could not be invited due to COVID-19-induced lockdown protocols. In addition, 2 participants were given the wrong stimulus set for the curries, such that in total only 16 participants had viable data for the TRF eating test (see section **2.6**).

3.6.1 –Fat Preference Scale

Fat preference was calculated by calculating the percentage of the total amount of fat eaten from the total amount of curry eaten, using the fat contents provided in Chapter II:

$$Fat\ Preference\ Scale = \frac{LF\ curry \times 0.015 + MF\ curry\ \times 0.045 + HF\ curry \times 0.1}{Total\ Curry\ Eaten} \times 100$$

This measure accounts for any differences in appetite due to other factors and focuses solely on the proportion of fat in the total food consumed. As the participants are left to complete their lunch for 30 minutes, they were able to return for second and third helpings as well as try any combination of the three curries. Figure 3.16 shows the distribution of the total amounts of each curry eaten by participants. The high-sugar low-fat curry (HSLF) does not appear to be as popular as the medium-fat medium-sugar (MFMS) or the high-fat low-sugar (HFLS) curry. This could be due to a discordance between the expected flavour of the curry and the high sugar concentration.

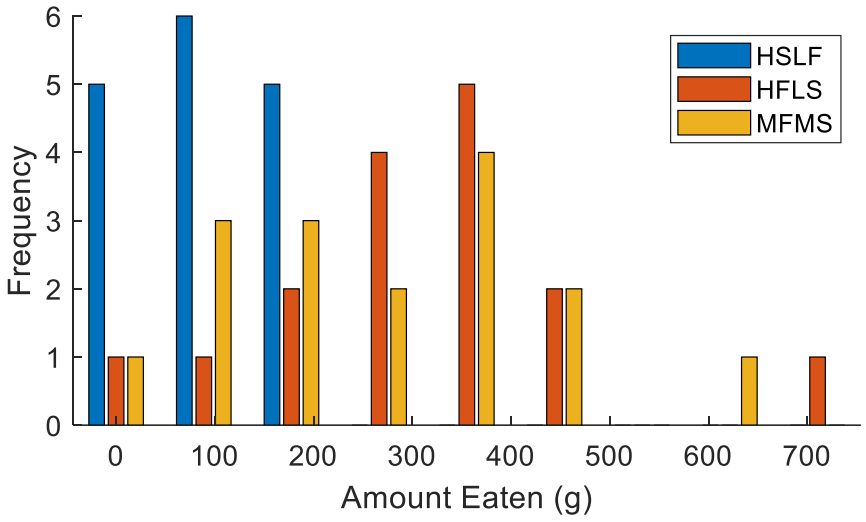

Figure 3.16. Distribution of the Different Types of Curry Eaten.

3.6.2 – Comparing Fat Preference between Experimental Task and Real Life

The first method of testing the ecological validity of the data was to establish if the subjective values that participants assign during the experiment are related to their real-life eating behaviour. Specifically, if psychophysical ratings and subjective value in the experiment were ecologically valid, the extent to which fat affects their subjective value of oral food stimuli should be related to their preference of fatty foods, leading to a higher fat preference scale. To that end, we used the betas obtained from the nutrient-based GLM

in section *3.4.2.2* to determine the extent to which fat content explains WTP. In specific, the beta for the fat content regressor was used as a proxy for the subjective value assigned to fat. Figure 3.17 shows that the individual betas associated with fat are indeed predictive of the participants' fat preference during the ad-libitum eating task (r = 0.566, p = 0.0223), thereby indicating that the subjective preference of fat during the milkshake tasting task carries across to a real-life eating task. More fundamentally, these results reinforce the notion that the values assigned in an experimental task such as the BDM do have implications for eating behaviour in real life, thereby implying that the results of our psychophysical and formal economic tasks are grounded in real-life preferences. Furthermore, the change in context from a sweet vanilla milkshake to a savoury curry implies that fat preference is maintained across these contexts as opposed to being confined to their specific contexts, thus implying that an individual who prefers a fatty savoury meal at lunch is also more likely to prefer a fatty, creamier milkshake.

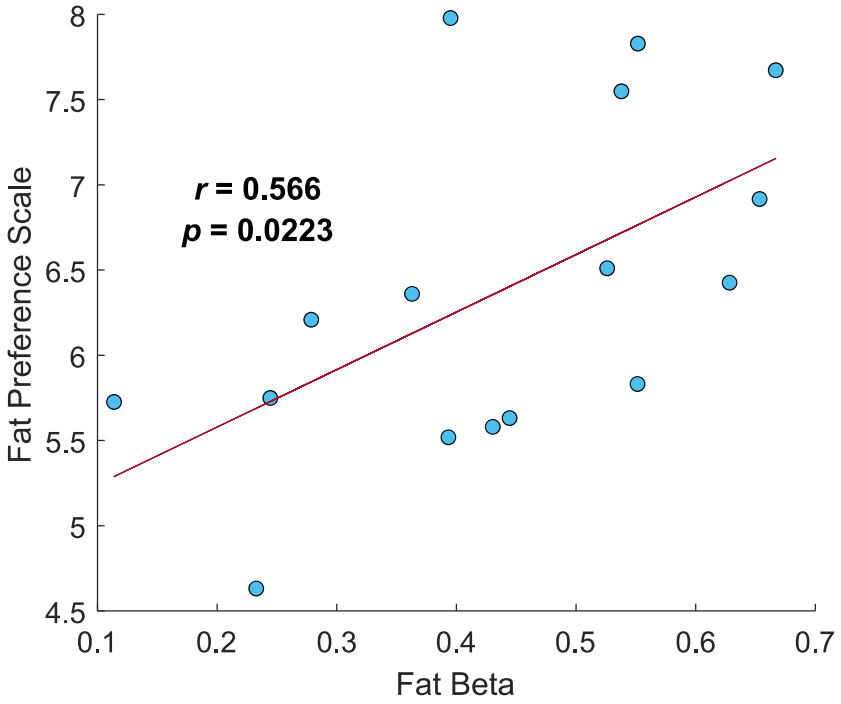

Figure 3.17. Plot of Individual Fat Preference Scale against Fat Betas from Macronutrient GLM.

3.6.3 –Fat Replacement in Real Life Eating

Another phenomenon that is of interest is the potential for fat replacement in actual food items. As previously observed in section **3.4**, there is a proportion of participants

susceptible to confusing increases in textural viscosity and protein with fat content. Meanwhile, there is also a substantial proportion whose fat detection is more finely tuned such that they are able to report that the control stimuli, such as the CMC stimulus, are lacking in fat. This is then reflected in their WTP ratings of the CMC stimulus. Therefore, it is possible to estimate the subjective value of fat replacement, that is, the ability of a non-caloric thickener like CMC to rescue the subjective value loss due to a lack of fat, through the WTP of the CMC stimulus.

As seen on Figure 3.18, there is a strong negative relationship between the subjective value for the CMC stimulus and the Fat Preference Scale (r = -.0729, p = 0.00135). These results imply that individuals who are more likely to accept non-fat viscous stimuli – that is, those who are more willing to pay for high-viscosity stimuli that do not contain fat – already do not consume much fat in real life. There are a multitude of implications that arise from this, as this means that those who are less likely to eat fatty stimuli in real life are the ones who are more susceptible to fat-replacement through the addition of non-caloric thickeners such as CMC. Therefore, dietary measures that try to reduce fat intake through the use of low-fat foods with added thickeners are targeting a population that already does not consume much fat, implying that more should be done to mimic the experience of consuming fat if the aim is to replace fat with non-caloric texture modifiers. In addition, such a strong negative relationship between the subjective value of the CMC stimulus and the Fat Preference Scale implies that those who are finely tuned to sensing fat and prefer it display fat-seeking behaviour in real life, where they identify foods that are high in fat and show a preference for that food over other macronutrients. The exact mechanisms behind this improved detection of fat should be explored more in order to establish what fat-replacement strategies might work on this population.

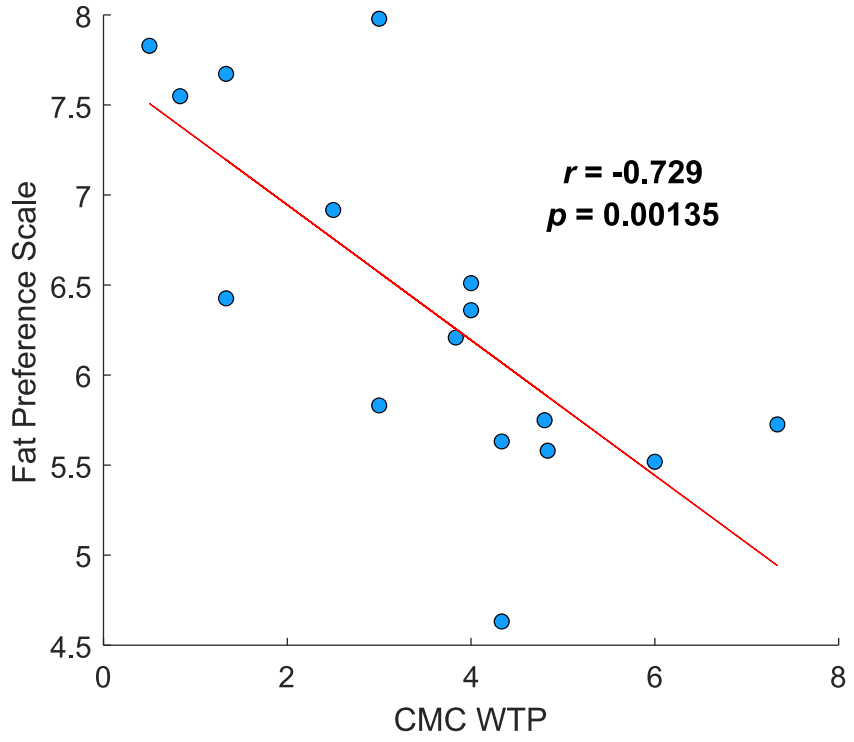

Figure 3.18. Plot of Fat Preference Scale against Individual Mean WTP for the CMC Stimulus.

3.7 – Summary

Overall, the behavioural results obtained both during pre-testing and post-testing provided insight into how subjective reward value is derived from sensory and nutrient properties of foods, specifically with regard to fat. From the psychophysical ratings, one notes that the sweetness ratings are associated with sugar content and the thickness and oiliness ratings are associated with fat content. Furthermore, there appears to be an interaction in all of these ratings such that the addition of sugar enhances thickness and oiliness perception, whereas the addition of fat increases perceived sweetness. This could be due to the fact that the stimuli were delivered in the form of milkshakes, and in daily life milkshakes that are higher in fat also tend to be higher in sugar, thereby eliciting some sort of association between these ratings.

The protein rating task indicates that protein is not well-sensed by individuals. Specifically, there is almost no difference between protein ratings of the high-protein stimulus and the low-protein stimuli, indicating that individuals are not able to identify the high-protein stimulus. Interestingly, the addition of protein does appear to rescue the subjective value that is otherwise lost due to a lack of fat, and the protein stimulus is

ranked as fat-containing by a substantial proportion of the participants. This indicates that humans may indeed have a mechanism for detecting added protein, although they are not necessarily specifically able to identify its addition as being protein per se, sometimes confusing it for fat content instead.

From the BDM task, one notes that the subjective values of the stimuli are highly variable among participants, although there does tend to be an overall trend to assigning higher values for stimuli that are higher in fat and sugar. Most crucially, this indicated that attempts at modelling the determinants of subjective value needed to be performed within each participant, due to the individual differences in subjective value for various stimuli. In doing so, the caloric load was found to be a predictor of subjective value, which confirms previous experiments on increasing subjective value through the modulation of caloric load without affecting taste (de Araujo et al., 2013).This also fits in with the notion that foods with greater caloric loads are more advantageous, which would explain an evolutionary pressure to prefer foods higher in calories. However, taking into account the macronutrient properties of the stimuli, especially the fat and sugar contents, provided a better explanation of the variance in subjective value. This echoes nutrient-specific feeding behaviours observed in both clinical samples and a general healthy population. Specifically, patients with mutations in the melanocortin-4 receptors overfeed specifically on fatty foods compared to healthy controls, while eating the same amount of sugary foods (van der Klaauw et al., 2016, 2014). Moreover, within the general population there appears to be a drive to fulfil a certain amount of protein threshold in one's daily food intake irrespective of total calorie intake (Martinez-Cordero et al., 2012; Simpson & Raubenheimer, 2005), a phenomenon also observed in various animal models (Skorupa et al., 2008; Sørensen et al., 2008). Therefore, while caloric load does play a factor in determining reward value, it appears that the specific macronutrient compositions of the foods provide a more complete picture of how the reward value of food is derived.

The improved macronutrient-specific model indicates that individual reward values for foods are related to their nutrient content, although this begs the question of how these macronutrient contents are sensed such that they are able to be integrated into a reward value for the stimulus. Therefore, we tested a model of the textural and taste parameters of the stimuli, including sugar concentration, viscosity and coefficient of sliding friction. This model marginally outperforms the macronutrient-specific model

that only contains fat and sugar, implying that it is just as if not more descriptive of how reward value is derived. However, the best model to predict reward value is the model that uses the trial-by-trial psychophysical ratings, specifically subjective sweetness and thickness. This is likely due to the fact that, while sweetness is correlated with sugar concentration and thickness is correlated with viscosity, this model takes into account trial-by-trial variations in intra-subject changes, such as attention, preparedness, adherence to tasting time length and overall satiety. Therefore, it appears that subjective reward value of oral food stimuli is derived from the macronutrient components of the stimuli, which are sensed through physical parameters and modulated by various endogenous factors that modulate the subjective perceptions of these stimuli and therefore the reward value.

While a clearer picture of the behavioural and psychophysical mechanics of determining reward value through nutrient content has been drawn, open questions still exist as to how this process occurs in the brain. More specifically, we know that structures such as the agranular insula respond to taste stimuli and the granular insula and oral somatosensory cortex respond to more somesthetic information. How this information is then processed when presented in conjunction, and where these structures forward information where it will be integrated into a reward signal for the food item is still largely unknown. Therefore, in Chapter IV, this thesis will continue to address these issues and specifically explore the neural structures that correspond to the formation of reward value of food from its nutrient composition.

Another interesting finding in this chapter has been the ability for both protein and CMC to partly rescue fat ratings in non-fat stimuli, specifically the bimodal nature of fat detection. Participants appeared to be divided into those who confuse the addition of protein or CMC for fat and those who are able to successfully report these as lacking in fat. Comparisons with real-life eating data indicate that those who assign greater subjective value to the CMC stimulus are less likely to consume fat in a real-life eating test, indicating that fat-replacement strategies through thickeners are more likely to succeed with people who already do not consume fat. However, this also opens up an avenue of research, as one may be able to see differences in neural processing between those who report these control stimuli as fatty and those who do not, which will also be explored further in the next chapter.

Chapter IV – fMRI Results

4.1 – Overview

Having established the behavioural and psychophysical components of oral food valuation in the previous chapter, we now turn to the neuroimaging data. Specifically, we are interested in how the neuroimaging data can inform us about how subjective reward values of food stimuli are formed. This chapter will first explain the specific analyses used, starting with the different approaches to first-level modelling and moving onto the second-level group analyses afterwards. The results of each analysis are then presented in the order of the effects studied. The effects of interest themselves follow the same structure as for the behavioural results in the previous chapter.

4.2 – Analyses

4.2.1 – First-Level Analyses

First-level analyses are analyses conducted within each participant. Due to the event-related design of the experiment, these are performed by modelling each event that the participant experiences. The first-level analyses indicate the within-participant voxel-based blood oxygen level-dependent (BOLD) responses, in contrast to the second-level analyses that examine the group-level activity (Penny, Friston, Ashburner, Kiebel, & Nichols, 2007). First-level analyses are conducted on pre-processed functional magnetic resonance imaging (fMRI) data (c.f. Chapter II) using either univariate general linear models (GLMs) or multi-voxel pattern analysis (MVPA).

4.2.1.1 – Univariate General Linear Models

Univariate GLMs involve modelling each event the participant experiences, such as the onset of the cue, the stimulus, the rinse and the rating periods. The results of the univariate GLMs are in the form of voxel-based betas for each regressor and each run, as well as contrast files for each regressor that collate voxel-level data across the runs. Univariate GLMs are performed on smoothed, normalised data (c.f. Chapter II) and measure the BOLD activity related to a specific regressor specified in the GLM. The results would then indicate regions of the brain that exhibit signed changes in BOLD responses related to the specified regressor or event (Friston, 2005). All univariate GLMs in this thesis were carried out using SPM12. We used a high-pass temporal filter with a cut-off period of 128s to remove low-frequency noise and slow drifts in the signal, which could

bias the estimates of the error. GLMs assuming first-order autoregression were applied to the time course of activation in which event onsets were modelled as single impulse response functions convolved with the canonical hemodynamic response function, with time derivatives included in the basis functions set. Unless otherwise specified, in all univariate GLM analyses in this thesis, the following event onsets were modelled as regressors:

- Cue
- Stimulus delivery
- Swallow cue
- First rating task
- Second rating task
- Rinse cue onset
- Rinse delivery
- Rinse swallow cue

For binary distinctions, such as the fat content rating, the stimulus delivery onset is divided into the two conditions, whereas continuous contrasts were entered into the stimulus delivery onsets as parametric modulators. We disabled the in-built orthogonalization procedure for successive parametric modulators in SPM in all analyses.

4.2.1.2 – Multivariate Pattern Analyses

Multivariate pattern analysis is a method to analyse the population-level differences in BOLD signals, focusing on the extent to which the pattern of activation differs in a specified area within the brain mask. Typically, MVPA yields more information and displays greater sensitivity to the encoding within functional neural architecture (Haxby et al., 2001; Norman, Polyn, Detre, & Haxby, 2006), as large inter-subject BOLD responses tend to result in weaker effects when observed using univariate GLMs. Furthermore, the questions answered by MVPA are less about which brain areas are more active during a certain task, such as ingesting high-fat foods, and more about the varying brain states in a specified area and how they correspond to – that is, encode – different types of information (Haxby, 2012). Evidence for discrete population-based encoding of taste stimuli in the orbitofrontal cortex (OFC) suggests that MVPA might be a more appropriate method to investigate this question in humans (Howard et al., 2015; Suzuki et al., 2017).

The MVPA analyses used in this thesis are performed using linear Support Vector Machines (SVMs) for categorical data, such as high-fat vs low-fat, and SVM regression for continuous data, such as the coefficient of sliding friction or the willingness-to-pay (WTP) values on The Decoding Toolbox (Hebart, Görgen, & Haynes, 2015). SVM has shown excellent sensitivity in classifying neural responses to visual stimuli (Cox & Savoy, 2003; Haxby et al., 2011) and olfactory stimuli (Howard et al., 2015) and is therefore an ideal candidate for the classification method. Using a leave-one-out cross-validation method, the classifier is trained on five scan runs within each subject and tested on the remaining run, repeated six times to obtain an average accuracy for the specified area.

The whole-volume MVPA is performed using a searchlight method on unsmoothed realigned data (c.f. Chapter II) in the subject native space. In doing so, a 9mm sphere was defined as a searchlight and used for MVPA, after which the searchlight moves onto the neighbouring voxel. Exploratory analyses with different sphere sizes suggested that a 9mm searchlight radius provided robust and accurate results, while small changes in sphere size did not fundamentally alter the results. The resultant accuracy maps are subsequently normalised onto the Montreal Neurological Institute (MNI) space, following the protocol used in similar MVPA studies (Howard et al., 2015; Kahnt, Heinzle, Park, & Haynes, 2011).

4.2.2 – Second-Level Analyses

Second-level analyses apply the results of the first-level analyses and perform formal statistical tests of significance on a group level.

4.2.2.1 – T-Tests

We used a one-sampled t-test on SPM12 against zero for both the univariate GLMs and the MVPA results. Using the univariate GLM output contrast file of interest allowed us to discern brain structures that exhibited a significant increase or a significant decrease in BOLD response to a specific contrast, such as stimulus contrasted with rinse. The results of the SVM analyses were accuracy maps that were subsequently normalised before being used in the t-tests, whereas the SVM regression outputs were z-score normalised correlation maps that were also normalised to MNI space. This approach echoes previous MVPA studies using subject native space (Howard et al., 2015; Kahnt et al., 2011).

4.2.2.2 - Conjunction Analyses

Conjunction analyses were used to formally test and identify the shared brain regions showing significant effects present in several maps. This analysis indicates that, in a significant portion of the subjects studied, this level of functional anatomy is significantly present in a number of participants. Although primarily used to compare shared regions in contrast files from univariate GLMs (Friston, Holmes, Price, Bü, & Worsley, 1999), conjunction analysis has also been used with accuracy maps from MVPA to indicate shared regions in taste encoding (Avery et al., 2020).

4.3.1 – Reporting of Results

4.3.1.1 – Thresholds and Parameters

In order to be included within the report, the relevant clusters of activations or accuracy values need to be significant at group-level in whole-brain corrections or small volume corrections (in the case of pre-defined regions of interest, see below). Specifically for whole-brain correction, the cluster (k_e) needs to be larger than 10 voxels (5 for small-volume corrections), in statistical maps thresholded at $P < 0.001$ (uncorrected), and satisfy the cluster-level p-value of 0.05 after family-wise error (FWE) correction. Notably, this is a substantially stricter threshold than similar alternatives, such as the False Discover Rate (FDR) correction in the case of smaller clusters (Chumbley & Friston, 2009), which in turn may lead to fewer results being reported (Nichols & Hayasaka, 2003). However, this approach would also imply greater confidence in the clusters being reported, especially those that span across smaller regions. Therefore, this cluster-cutting threshold was chosen.

4.3.1.2 – Small Volume Corrections

The areas that have been reliably shown to respond to oral fat stimuli and reward processing, as discussed in Chapter I, provide interesting points at which to start, as we expect effects to be seen in these regions. However, due to the strict cluster-cutting threshold, results that show in these regions may not always pass the threshold for whole-brain corrected results. Therefore, several Small Volume Corrections (SVCs) were applied in these areas of interest in order to formally test the effects found therein. The specific SVCs carried out used a spherical radius of 10mm for cortical areas and 6mm for subcortical areas, using a set of co-ordinates of interest from previous studies on oral taste stimuli. This approach follows previous fMRI studies on food rewards and decision-

making (Eldeghaidy et al., 2011; Grabenhorst, Rolls, et al., 2010; Rothemund et al., 2007; Seubert et al., 2015; Small et al., 2003).

Table 4.1 lists the coordinates used for SVCs in this chapter, which have been gathered from the most relevant previous studies. For the most part, this thesis will use specific coordinates as laid out here, as these studies were conducted specifically to explore the neural correlates of nutrient ingestion, specifically sugar and fat. However, one of the structures of interest, namely the oral SSC, varies considerably in its locations across studies. Previous studies, such as Grabenhorst and Rolls (2014) have attempted to review the literature on the oral SSC, finding that a large section of the SI has been found to be responsive to oral stimulation and averaged the coordinates of previous findings to [58, -14, 30] (see Table 4.1), although in that study peaks were found far posterior or inferior to these coordinates for different effects, such as [66, -18, 12] in the ingestion of pleasant fatty stimuli or [66, -22, 28] for fattiness ratings. Therefore, where sensible, one of these alternative coordinates may be used in the SVC.

Table 4.1

List of SVC Coordinates and References

Anatomical location	Co-ordinates			
	x	y	z	
Orbitofrontal Cortex (OFC)	32	34	-14	Grabenhorst, Rolls, Parris and d'Souza (2010)
Anterior Insula	36	10	10	de Araujo and Rolls (2004)
Midposterior Insula	48	-8	12	de Araujo and Rolls (2004)
Frontal Operculum	56	12	8	Grabenhorst, Rolls, Parris and d'Souza (2010)
Ventromedial Prefrontal Cortex (vmPFC)/Pregenual Cingulate	-2	44	-4	Clithero and Rangel (2013)
Hypothalamus	8	-8	2	Grabenhorst, Rolls, Parris and d'Souza (2010)
Amygdala	24	0	-12	Grabenhorst, Rolls, Parris and d'Souza (2010)
Ventral Striatum	12	2	0	Grabenhorst, Rolls, Parris and d'Souza (2010)
Oral Somatosensory Cortex (SSC)				
Averaged	58	-14	30	Grabenhorst and Rolls (2014)
Fattiness	66	-22	28	
Pleasantness	66	-18	12	

4.3.1.3 – Use of Delayed Onset

The necessarily long-lasting tasting period (7s) in the event-related design leads to the possibility that effects related to oral-sensing occurred after the typical haemodynamic response peak. In order to investigate effects that may occur after the initial stimulus delivery, we conducted parallel analyses using a 2.5s onset delay. Such delayed event onsets have been used in similar studies involving oral food stimuli with long event-related designs and extended tasting periods (de Araujo & Rolls, 2004; de Araujo et al., 2003; Small et al., 2003, 2004).

4.4.1 – Results

4.4.1.1 –Pump vs Rinse

As a starting point, we explored a simple contrast between all liquid reward stimuli and the rinse stimulus. This would give an indication of the main effects of oral food stimulus delivery, in contrast to receiving a tasteless isotonic solution. The results of both the univariate GLM (Table 4.2) and MVPA (Table 4.3) indicate that there are marked differences in processing between the experimental stimuli and the tasteless isotonic rinse. Due to the strong contrast between the presence of taste stimuli and the rinse, as well as the larger number of trials used, the results required a much stricter threshold. Therefore, the map was displayed at $p_{FWE\text{-}corr}<.05$ at peak level, after which clusters were compared.

As expected, the pump-rinse contrast yielded a large number of results, most notably the large activation cluster centred in the hypothalamus that extends across the ventral striatum and through to most of the anterior insula, in which one notes bilateral activation (Fig. 4.1). Further, one notes that there are activations that pass the threshold in areas such as the frontal operculum and, with appropriate SVCs, the orbitofrontal cortex and the oral SSC. These findings are echoed in the SVM analysis as well, with these areas showing high decoding accuracies. Interestingly, the SVM analysis also revealed high decoding accuracies in the midposterior and mid insula cortices, which did not register in the univariate GLM analysis. This observation is likely due to the different questions that canonical GLMs and MVPA answer, as it is likely that the midposterior and mid insula cortices do not experience an overall increase or decrease in BOLD signal upon stimulus delivery, but the pattern of individual voxel responses differ between the two conditions, leading to high searchlight decoder accuracy in the area.

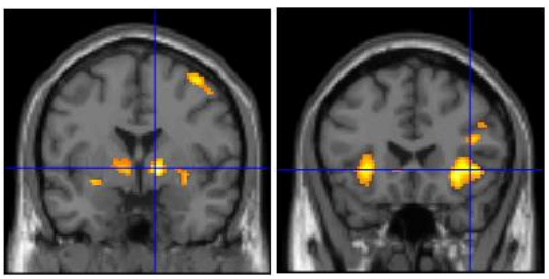

Figure 4.1. Univariate GLM activations in the hypothalamus extending into the striatum *(left)* and the anterior insula *(right)*.

Table 4.2

Univariate GLM Results of Pump-Rinse Contrast

Anatomical location	Co-ordinates x	y	z	$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
Stimulus Onset						
Positive Contrast						
Hypothalamus†	12	0	0	5.49	0.000	839
Dorsal dlPFC	46	28	26	5.18	0.000	306
Secondary Visual	-22	-90	-2	4.98	0.000	508
Hypothalamus	6	-20	-2	4.79	0.000	434
Cerebellum	2	-34	-32	4.78	0.011	155
Anterior Insula	-30	24	-2	4.75	0.001	234
Frontal Operculum	52	10	26	4.72	0.020	137
Hypothalamus	-8	8	-4	4.45	0.002	217
Secondary Visual	32	-84	-6	4.32	0.013	149
Supramarg Gyrus	-52	-20	36	4.30	0.007	170
Small Volume Correction at oral SSC [58, -13, 30]						
Oral SSC	62	-16	34	3.93	0.025	12
Small Volume Correction at OFC [32, 34, -14]						
OFC	28	28	-8	4.18	0.010	26
Negative Contrast						
Cerebellum	4	-62	-12	5.41	0.000	1005
Fusiform Gyrus	-40	-64	-20	4.48	0.000	295
2.5 s Delay						
Anatomical location	Co-ordinates **x**	**y**	**z**	$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
Positive Contrast						
Visuomotor Area	-20	-60	44	5.70	0.000	8624
Visuomotor Area	22	-52	42	5.32	0.000	1073
Premotor/Supp Motor	6	4	50	5.15	0.000	748
Hypothalamus	6	-24	-4	4.70	0.035	129
Anterior Insula	28	24	-6	4.51	0.001	266
Premotor/Supp Motor	52	4	36	4.47	0.000	671
Caudate	-6	0	8	4.43	0.000	521
Oral SSC	64	-16	32	4.40	0.000	441
Cerebellum	-6	-34	-32	4.33	0.000	407
Anterior Insula	-30	20	-4	4.23	0.013	159
Cerebellum	-6	-52	-26	4.23	0.013	159
Thalamus	12	-12	6	4.15	0.008	175
Anterior Cingulate	-4	-10	28	4.04	0.038	126
Fusiform	48	-64	-4	3.97	0.033	131
Negative Contrast						
No supra-threshold voxels survive at whole-brain level.						

† *This is part of a large cluster including most of the ventral striatum and anterior insula [30, 24 -4] and [40, 22, -2]*

Table 4.3

SVM Classification Results of Pump-Rinse Contrast

Stimulus Onset						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**Z**			
Sec Visual	-10	-100	-12	6.42	0.000	120
Anterior Insula	-22	20	2	6.41	0.000	216
Supramarg Gyrus	-56	-36	36	6.34	0.000	63
Visuomotor Area	4	-76	42	6.34	0.000	708
Midposterior Insula	-34	-12	8	6.24	0.000	105
Visuomotor Area	-4	-64	48	6.19	0.000	189
Cerebellum	-34	-54	-24	6.16	0.000	448
Medial OFC	12	24	-10	5.87	0.000	76
Frontal Operculum	56	8	4	5.86	0.000	127
Visuomotor Area	26	-66	42	5.79	0.000	70
Mid Insula	-36	4	6	5.54	0.000	25
2.5 s Delay						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
Visual Association	-42	-82	-6	6.39	0.000	698
Premotor/Supp Motor	-6	8	46	6.19	0.000	2008
Angular Gyrus	-56	-46	24	6.00	0.000	1404
Premotor/Supp Motor	34	2	50	5.95	0.000	1009
Oral SSC	-56	-18	20	5.93	0.000	824
Primary Motor Area	-34	-18	54	5.82	0.000	235

4.5 – Objective Regressors

4.5.1 – Calorie Content

Following the same line of reasoning in Chapter III, the first model tested on fMRI BOLD activations was that of caloric load. Food items with higher caloric loads should be more rewarding as the caloric load determines the amount of useful energy that can be obtained from the food item (Brunstrom et al., 2018). In fact, similar-tasting food items that have higher caloric loads have been reported to have a post-ingestive effect such that participants who were initially ambivalent towards them are more likely to select the higher calorie food items at follow-up (de Araujo et al., 2013). Hence, it stands to reason that caloric load of foods would be encoded in reward structures such that behavioural learning can occur. To that end, we modelled the caloric load of stimuli using a univariate GLM and SVR regression in order to specifically explore structures involved in the processing of caloric load.

A univariate GLM using calorie content as a regressor did not appear to show structures in the reward and sensory system that were positively correlated with the caloric load of the oral stimuli, either at the point of stimulus deliver or with a 2.5s delay (Table 4.4). In fact, BOLD levels in the left midposterior insula and oral SSC are negatively correlated with caloric load (Table 4.4, Fig. 4.2), whereas the SVR regression highlighted population encoding of the caloric load in the right oral SSC. This decoding accuracy may be owing to the fact that some control stimuli, such as the CMC stimuli, are somesthetically more similar to fatty stimuli whilst containing a much lower number of calories, such that sensory areas respond to these textural properties.

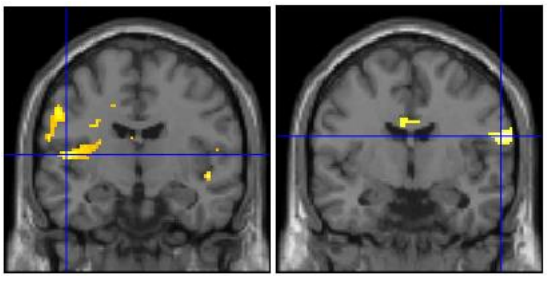

Figure 4.2. *Left* Negative contrast of caloric load in the midposterior insula (y=0) *Right* SVM regression decoding of caloric load in the oral SSC (y=-6)

The abovementioned findings seemingly indicate that calorie density is not necessarily linked to specific reward structures. While caloric load has been shown to

modulate preference in previous studies (de Araujo et al., 2013), this effect is more likely to be due to post-ingestive satiety signalling through satiety-related hormonal signalling well after the point of ingestion.

Table 4.4

Univariate GLM Results of Caloric Load Contrast

Stimulus Onset						
Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	**y**	**z**			
Positive Contrast						
Primary Motor Area	-32	-24	62	4.93	0.000	310
Visuomotor Area	-28	-52	52	4.69	0.001	239
Visuomotor Area	16	-66	60	4.68	0.003	184
Negative Contrast						
Superior Temp Gyrus	-36	-44	12	4.79	0.000	311
Primary Motor Area	-48	-10	8	4.60	0.001	207
Mid Insula	-58	0	16	4.50	0.000	320
2.5 s Delay						
Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	**y**	**z**			
Positive Contrast						
Primary Motor Area	-32	-24	64	5.67	0.000	903
Visuomotor Area	24	-58	54	5.34	0.000	2833
Cerebellum	32	-38	-32	4.03	0.020	117
Negative Contrast						
No supra-threshold voxels survive at whole-brain level.						

Table 4.5

SVM Regression Results of Caloric Load Values

Stimulus Onset						
Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	**y**	**z**			
No supra-threshold voxels survive at whole-brain level.						
Small Volume Correction at oral SSC [62, -10, 20]						
Oral SSC	62	-6	20	4.19	0.001	140
2.5 s Delay						
Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	**y**	**z**			
Visuomotor Area	-30	-68	48	4.87	0.000	677
Visuomotor Area	10	-50	58	4.64	0.020	267
Angular Gyrus	-48	-64	28	4.10	0.001	450

4.5.2 – Nutrient Content

In light of the results and conclusions of Chapter III, where the determinant factors behind subjective reward value do not seem to be limited to solely the caloric content of the food item being tasted, we used models with specific macronutrient (that is, sugar and fat) levels as regressors, which explained the variance of subjective reward much better than models using simple caloric load, thereby suggesting that there is a specific drive towards the consumption of certain macronutrients that is reinforced through the reward system. Indeed, neural reward systems have also been shown to encode specifically the macronutrient of food items even from visual stimuli (Suzuki et al., 2017). Therefore, modelling the macronutrient components of the real-time orally delivered stimuli would show how the specific nutrient components are sensed and the mechanisms by which their ingestion contribute to a reward value.

4.5.2.1 – Fat

Fat is a highly rewarding macronutrient. In addition to being more calorically dense than other macronutrients, fat also appears to elicit greater hedonic responses during ingestion, especially when paired with consonant flavours (Grabenhorst, Rolls, et al., 2010). This specific rewarding characteristic of fat is still largely unexplored, particularly in the context of the mechanisms involved in its sensing and how it contributes to reward value. Thus, modelling neural responses to the ingestion of high-fat stimuli would elucidate the neural processes behind fat-sensing as well as how fat contributes to reward valuation.

Notably, a univariate GLM does not indicate any clusters above the threshold for significance. However, MVPA would identify areas that have distributed pattern encoding of fat content. Due to the distribution of fat content in the stimulus set being binary, at either 0g or 36g of fat, an SVM classifier was used. The results of this analysis (Table 4.6) highlighted the oral SSC and midposterior insula as areas encoding oral fat (Fig. 4.3). Furthermore, an SVC at the vmPFC also implicates this regions in fat processing, with mid- and midposterior insular effects sustained up to the 2.5s delay.

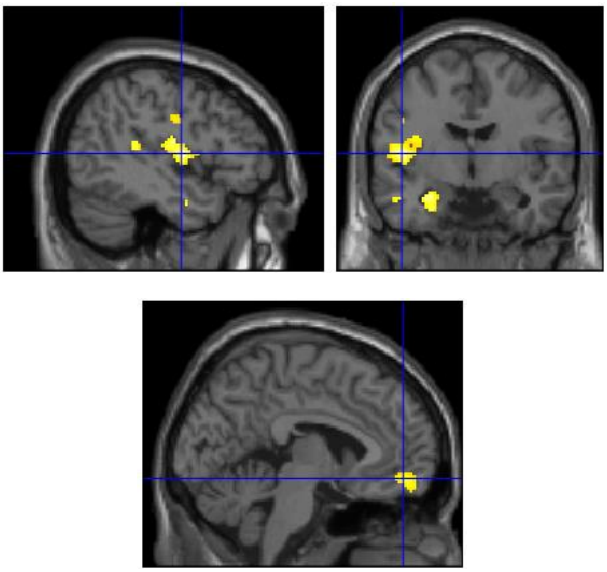

Figure 4.3. *Top* Oral SSC and midposterior insula encoding of oral fat content. *Bottom* vmPFC encoding of oral fat content

Taken together, these findings indicate that oral fat content is reflected in both the oral SSC/midposterior insula area as well as the vmPFC. As the midposterior insula and the adjacent oral SSC both respond to somesthetic information as opposed to taste stimuli (Grabenhorst & Rolls, 2014; Pritchard et al., 1986), it is likely that this is in response to the difference in textural properties of the oral stimuli, such as the viscosity and the CSF. The vmPFC, however, is known to represent the combined subjective economic value of stimuli (Kahnt et al., 2011; Plassmann et al., 2007). Therefore, this result in particular may reflect the higher economic value assigned by participants to the high-fat stimuli in the stimulus set.

Table 4.6

SVM Classification Results of Fat Content

Stimulus Onset						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**Z**			
Oral SSC	-46	-6	8	4.27	0.020	402
Small Volume Correction at vmPFC [-2, 44, -4]						
vmPFC	-4	50	-10	3.49	0.012	34
2.5 s Delay						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
No supra-threshold voxels survive at whole-brain level.						
Small Volume Correction at left midposterior insula [-46, -6, 8]						
Midposterior Insula	-50	-2	0	4.17	0.007	60

4.5.2.2 – Sugar

Sugar has been widely reported to be an appetitive and rewarding stimulus (G. K. W. Frank et al., 2008; A. A. Lee & Owyang, 2017; Wolf, Bray, & Popkin, 2008). Therefore, investigating how sugar is encoded in the reward system might be able to shed more light on how this occurs. However, the univariate GLM did not appear to indicate any supra-threshold clusters, and MVPA results were also lacking in overly strong clusters in the areas of interest, although with a 2.5s delay areas such as the midposterior and the mid insula appeared to show sugar-encoding properties. This may indicate that sugar processing may occur with a slight delay, which explains the delayed onset in long event-related designs. Furthermore, it is possible that our design, which was optimized for investigating fat and texture effects, with the use of only two low-sugar stimuli (HFLS and LFLS) out of the seven total stimuli may have reduced the power to provide a meaningful contrast of various sugar levels, which may have been improved by diversifying the sugar concentration of the stimuli.

Table 4.7

SVM Classification Results of Sugar Content

Stimulus Onset						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**Z**			
Primary Visual Area	14	-70	6	4.05	0.001	682
2.5 s Delay						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
Ventral Post Cingulate	8	-52	26	5.10	0.000	1034
Supramarg Gyrus†	-50	-32	38	4.52	0.002	670
Small Volume Correction at right midposterior insula [48, -8, 12]						
Mid Insula	52	-4	4	3.42	0.022	10

† *This is a large section that extends into the left mid-insular cortex [-58, -12, 12]*

4.5.3 – Physical Texture Parameters

Physical texture parameters provide the candidate mechanisms through which nutrients, including fat, are sensed. Specifically in the context of fat, the exact mechanisms of detection is disputed, although there is currently more evidence for fat detection to occur through the sensing of the textural properties of foods than through taste (de Araujo & Rolls, 2004; B. V. Kulkarni & Mattes, 2014; E. T. Rolls et al., 2018; Running et al., 2013). Therefore, this section will discuss both univariate GLM and MVPA results for textural parameters in order to shed light on how neural processing for these parameters occurs.

4.5.3.1 – Viscosity (log values)

One of the textural parameters crucial for fat detection is the viscosity of oral stimuli. Fluids with higher fat contents tend to be more viscous than those with lower fat contents. As up until the recent few decades viscosity had been the focal textural parameter pertinent to fat content, various viscosity series have been used in studies exploring the textural representations of fat (de Araujo & Rolls, 2004; Grabenhorst, Rolls, et al., 2010). Hence, modelling the neural responses to viscosity would identify brain structures involved in the encoding of this particular textural correlate of fat.

A univariate GLM with the log of the viscosity values in centipoise (cP) of each stimulus as a regressor highlights a large cluster in the SI of the left hemisphere, where the cluster peak is located between the primary somatosensory and primary motor cortex and the cluster reaches posteriorly towards the left supramarginal gyrus. While this area is more superior than our usual criteria for the oral SSC, as the SI is arranged in such a way that the orofacial responsive structures are mapped more inferiorly than other body parts, the cortical area onto which somesthetic stimuli in the oral cavity are mapped is widely spread (de Araujo & Rolls, 2004; Eldeghaidy et al., 2011; Miyamoto et al., 2006; Pardo, Wood, Costello, Pardo, & Lee, 1997; Veldhuizen et al., 2011), such that this activation is still reasonably within the oral SSC. We also note that viscosity is negatively correlated with activation in the left midposterior insula and oral SSC, inferior to the responses noted in the positive contrast. These results imply that overall the inferior sections of the oral SSC and the left midposterior insula are negatively tuned to viscous stimuli in the oral cavity, whereas the superior section of the oral SSC appears to have more neurons that are tuned to increasing viscosity of oral stimuli. These results appear to be in contrast with previous work showing that increasing viscosity elicits

greater BOLD activations in the midposterior insula and anterior insula, although that study used a pure viscosity series without additional flavour or nutrient components (de Araujo & Rolls, 2004). Also interestingly, previous studies have shown the inferior section of SI, otherwise termed the oral SSC, to positively correspond to viscosity or fatty texture (Grabenhorst & Rolls, 2014), our findings indicate that a more superior area of the SI may be more likely to be positively tuned to increasing viscosity.

The results of the SVM regression also indicate other areas that are not indicated in the univariate GLM. Of note, we see a different balance of decoding strengths, indicating that the area with the most predictive BOLD responses of oral stimulus viscosity, as indicated by statistical significance, is the lateral OFC (Table 4.9, Fig. 4.4). This indicates that the OFC as a whole structure may not be tuned either positively or negatively to viscosity, although individual or neighbouring neurons may in fact encode viscosity such that we are able to predict the viscosity of the oral stimuli from the pattern of BOLD activations therein. Furthermore, the SVM regression analysis also implicates the right midposterior insula and the oral SSC in the processing of oral viscosity (Table 4.9, Fig. 4.4), although the univariate GLM only highlights the left midposterior insula and oral SSC (Table 4.8), thereby implying a more population-based encoding in the right oral SSC and right midposterior insula. The amygdala is also shown to encode the viscosity of oral stimuli (Table 4.9, Fig. 4.4), which is not present in the univariate GLM contrasts. In addition to the aforementioned key structures, various motor and visual structures were also responsive to viscosity, although the visual structures likely encoded the unique visual cues associated with each stimulus, whereas the motor structures might reflect the difference in effort required for lingual movement for more viscous stimuli.

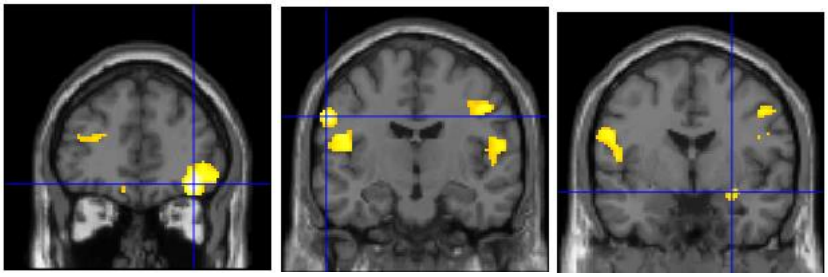

Figure 4.4. *Left* MVPA results showing a large viscosity-encoding cluster in the OFC (y = 42). *Middle* MVPA results showing viscosity encoding in the bilateral midposterior insula and oral SSC (y = -12). *Right* MVPA results showing viscosity encoding in the amygdala (y = -2)

The results of the viscosity univariate GLM and MVPA paint an overall picture in which the oral SSC, especially the left oral SSC, and the midposterior insula have more broad representations of the viscosity of oral stimuli, in that voxels in a particular segment are overall either tuned positively or negatively to viscosity, whereas the OFC appears to have a more distributed encoding where the pattern of activation of individual voxels encodes viscosity in the oral cavity. Both of these regions have been implicated in fMRI studies using canonical univariate GLMs (de Araujo & Rolls, 2004; Grabenhorst & Rolls, 2014; Grabenhorst, Rolls, et al., 2010), and the viscosity encoding of the amygdala echoes both fMRI and single-neuron primate studies on oral fat stimuli (Eldeghaidy et al., 2011; Kadohisa, Verhagen, et al., 2005). The use of MVPA in addition to univariate GLMs, however, has shed light on the nature of the encoding within these structures.

Table 4.8

Univariate GLM Results of Viscosity Contrast (Using Log Values)

Stimulus Onset						
Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	**y**	**z**			
Positive Contrast						
Oral SSC†	-32	-24	64	4.92	0.000	311
Visuomotor Area	16	-66	60	4.68	0.003	180
Visuomotor Area	-28	-52	52	4.67	0.001	237
Negative Contrast						
Medial Temp Gyrus	-36	-44	12	4.83	0.000	306
Midposterior Insula	-48	-10	8	4.66	0.004	174
Oral SSC	-58	0	16	4.47	0.000	287
2.5 s Delay						
Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	**y**	**z**			
Positive Contrast						
Primary Motor	-36	-24	54	5.60	0.000	846
Visuomotor Area	24	-58	54	5.41	0.000	2663
Cerebellum	32	-36	-32	3.94	0.010	134
Negative Contrast						
No supra-threshold voxels survive at whole-brain level.						

† *This large cluster includes the left primary motor area and the supramarginal gyrus.*

Table 4.9

SVM Regression Results of Viscosity Values (Using Log Values)

Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	y	z			
Stimulus Onset						
OFC	38	42	-14	4.68	0.000	745
Premotor/Supp Motor	12	22	54	4.45	0.003	381
Cerebellum	-32	-62	-28	4.42	0.002	402
Primary Motor Area	-60	-12	34	4.31	0.000	900
Small Volume Correction at oral SSC [62, -10, 22]						
Oral SSC	54	-10	18	3.92	0.008	44
Small Volume Correction at amygdala [22, 0, -18]						
Amygdala	26	-2	-16	3.46	0.004	32
Small Volume Correction at left anterior insula [-36, 12, 6]						
Operculum	-42	6	8	3.48	0.002	17
2.5 s Delay						
Inferior Temp Gyrus	-42	-4	-44	4.94	0.001	514
Primary Motor Area	56	-12	42	4.92	0.000	3137
Supramarg Gyrus	-44	-26	20	4.83	0.000	2426
Cerebellum	20	-64	-20	4.71	0.000	960
Visuomotor Area	36	-46	50	4.61	0.000	1306
Angular Gyrus	-56	-64	10	4.54	0.003	393
Small Volume Correction at ventral striatum [12, 2, 0]						
Hypothalamus	8	0	2	3.28	0.013	5

4.5.3.2 – Coefficient of Sliding Friction

Another important property of fatty stimuli is their lubricative texture in relation to non-fatty stimuli. The role of tribology in fat sensing and reward has largely been unexplored, in the context of human neuroimaging. This textural property is formally quantified through the change in the oral coefficient of sliding friction (CSF), as measured using the custom tribometer described in Chapter II. While viscosity and CSF are negatively linked in most stimuli, the decrease in CSF and increase in viscosity is disproportional such that modelling neural activity in relation to the CSF is likely to yield new information compared to the viscosity models

Interesting univariate GLM findings arise when using the CSF of each stimulus as a parametric modulator for the stimulus delivery regressor. Of note, a positive contrast identifies bilateral oral SSC activation correlated with an increase in CSF (Fig. 4.5, *Top*). Interestingly, a negative contrast indicated that a decrease in CSF is correlated with activation in a more superior location of the SI (Fig. 4.5, *Middle*), which is notably closer to the average of coordinates used by Grabenhorst and Rolls (2014) drawn from various previous studies on the oral SSC (de Araujo & Rolls, 2004; Eldeghaidy et al., 2011; Miyamoto et al., 2006; Pardo et al., 1997; Veldhuizen et al., 2011), albeit in the left hemisphere. As univariate GLMs analyse smoothed data, this seems to indicate that more neurons in the right oral SSC are, on a population level, positively tuned to oral CSF, whereas a greater population of neurons in the left oral SSC encode decreasing CSF. In order to investigate this phenomenon further, we therefore turn to the MVPA results, where a cluster at the oral SSC is observed at a location inferior to that of the collated previous results, adjacent to the mid-insula (Fig. 4.5, *Bottom*). This may be due to the highly detailed mapping of oral somesthetic input in the SI, thereby leading to a large area of representation that relies on population encoding and is, therefore, more difficult to observe with the smoothed data on the univariate GLM.

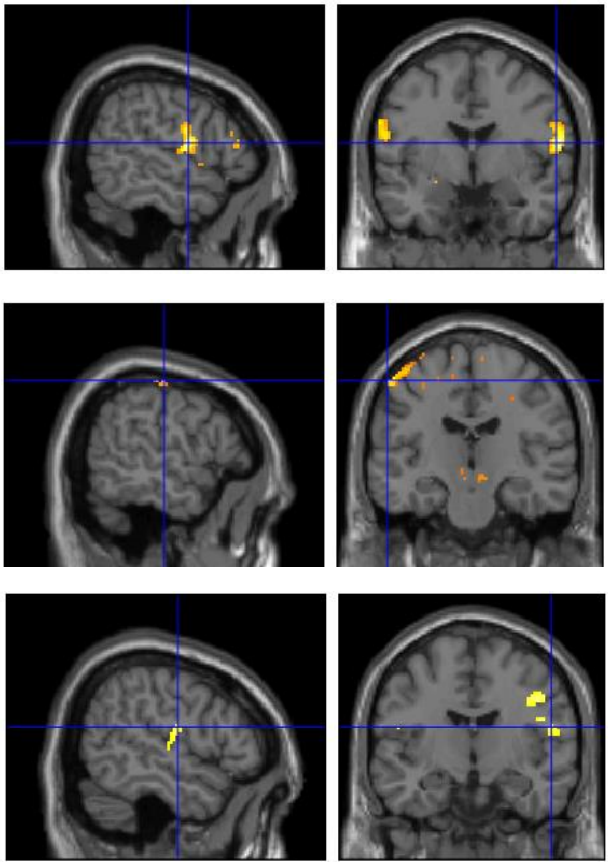

Figure 4.5. *Top* Positive contrast of oral stimulus CSF in oral SSC. *Middle* Negative contrast of oral stimulus CSF in left oral SSC. *Bottom* MVPA results showing encoding of oral stimulus CSF in oral SSC.

The distinction between MVPA and univariate GLM analyses was also present in in the OFC responses to the CSF of oral stimuli. Figure 4.6 displays this difference in overall BOLD signal (from the univariate GLM) and the voxel-level pattern of activations (from the SVM regression) in the OFC, which appears to respond to decreasing oral CSF only weakly from the negative contrast, although the MVPA analysis highlights a substantial effect, indicating that, while the OFC overall might not undergo a strong BOLD response to decreasing CSF, the pattern of encoding in the OFC does indeed reflect CSF within the oral cavity, suggesting more distributed encoding of CSF in the OFC. Importantly, this finding is the first time that oral CSF as a textural parameter has been shown to modulate OFC activation in humans, echoing recent similar findings in the role of the primate OFC in encoding CSF (E. T. Rolls et al., 2018). Previous findings in primates have differentiated between neurons that respond strictly to viscosity and other fat-responsive neurons whose responses are independent to the viscosity of oral food stimuli

(Kadohisa, Rolls, et al., 2005; E. T. Rolls et al., 1999). It is likely that signal in the OFC that arise due to fat but are not explained by viscosity may indeed be signals related to CSF.

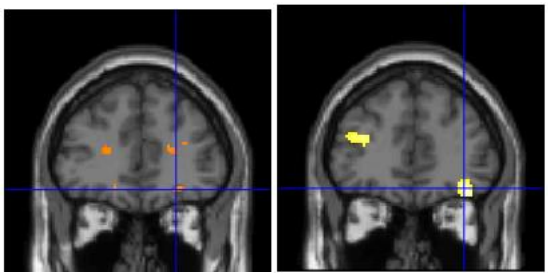

Figure 4.6. *Left* Negative contrast of oral stimulus CSF in the OFC (y = 40). *Right* MVPA results showing more prominent encoding of oral CSF in the OFC (y = 42).

Similar to the viscosity models, the models comparing neural responses to CSF have implicated, among others, the oral SSC and the lateral OFC as areas responsible for the encoding of CSF. As the oral SSC is known to respond to somesthetic stimuli in the oral cavity, and the OFC is known to respond to the presence of fat in the oral cavity, it is likely that these structures encode the textural parameters that correspond to oral fat stimuli, namely viscosity and CSF.

Table 4.10

Univariate GLM Results of CSF Contrast

Stimulus Onset						
Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	**y**	**z**			
Positive Contrast						
Oral SSC	58	-2	14	5.10	0.000	214
Cerebellum	22	-64	-22	4.67	0.000	1353
Cerebellum	-38	-74	-32	4.64	0.000	420
Cerebellum	-8	-76	-38	4.52	0.017	109
Oral SSC	-50	-12	36	4.46	0.000	269
Ventral Post Cingulate	24	-52	24	4.34	0.028	99
Ventral Post Cingulate	12	-52	30	4.30	0.000	432
Secondary Visual	-4	-98	4	4.25	0.002	77
Cerebellum	32	-74	-34	4.24	0.000	261
Angular Gyrus	52	-70	30	3.99	0.015	113
Small Volume Correction at right frontal operculum [56, 12, 8]						
Frontal Operculum	60	8	0	3.48	0.050	5
Negative Contrast						
Oral SSC	-56	-18	56	5.61	0.000	455
Supramarg Gyrus	-32	-40	48	4.73	0.014	114
Hypothalamus	8	-2	-2	4.46	0.000	245
Oral SSC	46	-36	62	4.30	0.003	152
Dorsal ACC	-14	32	10	4.24	0.037	93
Small Volume Correction at right OFC [32, 34, -14]						
OFC	23	40	-14	3.63	0.022	13

2.5 s Delay						
Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	**y**	**z**			
Positive Contrast						
Cerebellum	-16	-60	-16	5.63	0.000	662
Oral SSC	60	0	14	4.83	0.000	284
Premotor/Supp Motor	-52	-6	30	4.19	0.001	171
Cerebellum	34	-76	-32	4.14	0.000	538
Negative Contrast						
Oral SSC	-48	-30	64	5.11	0.000	708
Visuomotor Area	28	-48	58	5.08	0.034	94
Supramarg Gyrus	-58	-24	32	4.45	0.006	133
Anterior Insula	28	26	2	4.41	0.006	133
Premotor/Supp Motor	-48	2	26	4.21	0.001	187
Supramarg Gyrus	62	-22	34	4.17	0.001	177
Premotor/Supp Motor	10	10	50	3.95	0.037	92
Premotor/Supp motor	-18	-2	52	3.91	0.005	136
Small Volume Correction at ventral striatum [12, 2 0]						
Ventral Striatum	20	2	-4	4.41	0.004	36

Table 4.11

SVM Regression Results of CSF Values

Stimulus Onset						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
Angular Gyrus	40	-47	32	4.15	0.000	640
Small Volume Correction at OFC [32, 34, -14]						
OFC	36	42	-16	4.08	0.012	30
Small Volume Correction at right midposterior insula [48, -8, 12]						
Oral SSC	54	-10	18	3.46	0.009	40
Small Volume Correction at left midposterior insula [-48, -12, 6]						
Oral SSC	-52	-16	14	3.23	0.025	12
2.5 s Delay						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
Sec Visual	20	-72	-20	3.99	0.005	341
Inferior Temp Gyrus	-42	-2	-42	3.98	0.013	285
Oral SSC	56	-14	36	3.84	0.003	374
Small Volume Correction at right midposterior insula [48, -8, 12]						
Mid Insula	52	-10	20	4.45	0.006	56

4.5.3.3 – Conjunction of Sliding Friction and Viscosity

Both CSF and viscosity represent the textural parameters typically associated with fat. Thus, areas that process them in conjunction combine both the viscosity and CSF information to relay information about fat content. By formally testing this possibility using a conjunction analysis of the SVM regression results of both parameters, we see that viscosity and CSF share a large number of structures in their processing (Table 4.12). Most notably, the large clusters in the lateral OFC and the oral SSC, as well as the midposterior and posterior insular cortices are shown to be shared structures involved in both viscosity and CSF encoding (Fig. 4.7). In addition, various other structures in the reward system are also implicated, such as the dorsal anterior cingulate cortex (ACC).

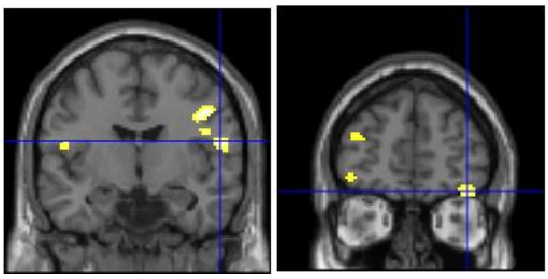

Figure 4.7. *Left* Conjunction of viscosity and CSF in the oral SSC/posterior insula *Right* Conjunction of viscosity and CSF in the OFC

The shared structures involved in the processing of both viscosity and CSF indicate that these two textural parameters are closely linked with each other. This is much more notable in areas such as the oral SSC and the mid- to midposterior insula, which are areas known to encode textural and somesthetic information about the oral cavity. The overlap in the OFC is also highly interesting, as this implies that the OFC encodes both of these textural parameters. Due to the multiple sensory inputs into the OFC (Barbas, 2007; McCabe & Rolls, 2007), as well as its responsiveness to oral fat stimuli (Grabenhorst, Rolls, et al., 2010; E. T. Rolls, 2011; E. T. Rolls et al., 1999), it is likely that the OFC does indeed encode fat content through the textural parameters as processed by the oral SSC and midposterior insula.

It is nonetheless important to point out that not all areas that show either viscosity or CSF encoding are present in this conjunction analysis, which suggests that the observed conjunctions are not simply driven by the intercorrelation of the two parameters. Therefore, the brain structures presented in the conjunction analysis are the structures that encode both viscosity and CSF of oral food stimuli.

Table 4.12

Conjunction Results of Log Viscosity and CSF

Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	y	z			
Oral SSC	46	-8	38	4.24	0.000	202
Angular Gyrus	38	-76	36	4.21	0.000	638
Cerebellum	-30	-60	-32	4.19	0.000	101
Posterior Insula	56	-8	18	4.15	0.000	163
Visual Association Area	-16	-78	36	4.01	0.000	420
Supramarg Gyrus	42	-48	44	3.98	0.000	197
Cerebellum	-24	-86	-40	3.89	0.000	249
Lateral OFC	38	50	-18	3.86	0.000	121
Dorsal ACC	-2	6	42	3.79	0.008	69
Midposterior Insula	-50	-12	16	3.78	0.000	142
Cerebellum	10	-56	-50	3.75	0.003	78
Anterior PFC	-36	46	20	3.60	0.003	78

4.6 – Behavioural Ratings

In Chapter III, it was established that the best determinants of subjective reward value were the trial-by-trial subjective psychophysical ratings of each stimulus. The large improvement in explanatory power of the model is likely due to the fact that this model takes into account trial-by-trial fluctuations in intra-participant internal states, such as attention and satiety, in addition to inter-participant differences such as sensitivity to sugar and fat. Therefore, in this section we model the neural responses to the various subjective ratings provided by participants to explore the neural mechanisms involved in such processing.

Due to the design of the study – that is, half the trials asked for fat content rating and BDM and the other half asked for sweetness and thickness ratings – the results of most of the subjective rating models used the average values the participants have for each stimulus, in order to ensure that all the trials were used,

4.6.1 – Subjective Value

Subjective value was measured using a modified version of the BDM task (see Chapter II), where the willingness to pay for each stimulus provided an incentive-compatible proxy for the participant's absolute value of the tasted stimulus. Therefore, the modelling of BDM values would identify neural structures that encode the subjective value of oral food stimuli.

Although a univariate GLM does not yield any supra-threshold clusters, an SVM regression analysis highlights the ventral posterior cingulate and the OFC as areas that encode the subjective reward of oral food stimuli at the point of ingestion (Fig. 4.8, *Top*). In addition, after a 2.5 s delay the univariate GLM also identifies the vmPFC (Fig. 4.8, *Bottom*) and the anterior insula as areas that encode the subjective value of oral food stimuli, in addition to the sustained encoding in the OFC. The role of the OFC in subjective and reward value processing has been documented in both primates and humans, especially in the case of food stimuli (Grabenhorst & Rolls, 2014; Padoa-Schioppa & Assad, 2006; Peters & Büchel, 2010; E. T. Rolls & McCabe, 2007), a notion that is further strengthened by the MVPA results in this study. Furthermore, the vmPFC also encodes subjective value, which is consistent with previously established literature using formal economic measures (DiFeliceantonio et al., 2018; Kahnt et al., 2011; Peters & Büchel, 2010). Notably, this effect only seems to occur after a 2.5s delay of the onset, implying the

processing and encoding in the vmPFC may occur later than in the posterior cingulate and the OFC.

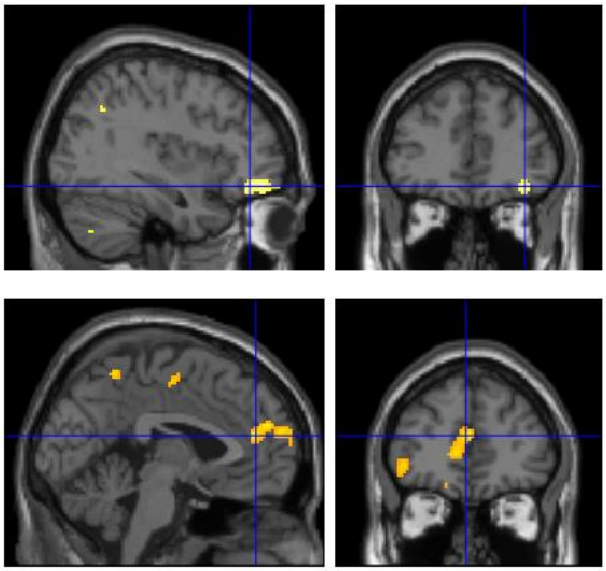

Figure 4.8. *Top* Encoding of subjective value in the OFC during stimulus delivery *Bottom* Encoding of subjective value in the vmPFC/pACC with a 2.5s delay

Table 4.13

SVM Regression Results of BDM Values

Stimulus Onset						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
Ventral Post Cingulate	12	-52	22	3.85	0.029	225
Small Volume Correction at OFC [32, 34, -14]						
OFC	38	40	-14	3.83	0.010	34
2.5 s Delay						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
Premotor/Supp Motor	-18	-14	52	4.95	0.019	257
Prim Auditory	-54	-18	2	4.25	0.041	213
Cerebellum	18	-54	-26	4.25	0.003	376
Insula	36	4	8	4.22	0.004	361
Premotor/Supp Motor	-42	-10	46	4.19	0.001	438
Primary Motor	42	-12	36	4.13	0.001	496
dlPFC/vmPFC	-2	52	24	3.77	0.004	352
Small Volume Correction at OFC [32, 34, -14]						
OFC	36	40	-10	3.17	0.040	3

4.6.1.2 – Conjunction of Viscosity, Sliding Friction and BDM

In order to explore the shared areas between viscosity, CSF and subjective value, we conducted a conjunction analysis of the three results. In doing so, areas that potentially encode all three variables, that is, textural parameters as well as subjective reward value, would be identified, which would indicate the convergence of textural stimuli and their integration into a subjective value.

Although no clusters survive the whole-brain correction (Table 4.14), an SVC at the OFC indicates that the OFC is involved in the processing of both fat-related textural parameters and subjective reward value (Fig. 4.9). Notably, while the cluster identified through SVC is the one under the crosshairs, the OFC effect extends to a larger cluster further anterior. This identifies the OFC as the unique structure that holds representations of both textural information and reward value, which implies that this is where textural information is likely integrated into a whole reward value for the stimulus.

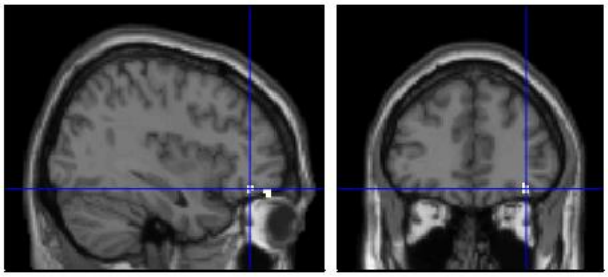

Figure 4.9. Conjunction of CSF, viscosity and BDM models in the OFC.

Table 4.14

Conjunction of CSF, Viscosity and BDM Accuracy Maps

Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	y	z			
No supra-threshold voxels survive at whole-brain level.						
Small Volume Correction at OFC [32, 34, -14]						
OFC	38	40	-16	3.41	0.017	10

4.6.2 – Thickness

As explained in Chapter III, the subjective thickness ratings are correlated with the viscosities of the stimuli themselves. Therefore, the thickness ratings can be thought of the subjective perception of the viscosity of the stimuli. Following this reasoning, modelling the thickness ratings of each stimulus, on a trial-by-trial basis, would be likely to be able to reflect both inter-participant variability, in terms of being able to sense the actual differences in viscosity, and intra-participant variability, due to the use of the online thickness ratings of the stimuli in the univariate GLM.

The univariate GLM analysis indicates that the amygdala positively encodes the perceived thickness of the stimuli. The negative contrast, however, highlights a large area with a peak at the posterior cingulate that extends bilaterally past the primary and premotor areas through to the primary sensory area, in addition to areas in the dlPFC and the vmPFC. Both these results are maintained even in the 2.5s delay model, indicating that these effects are sustained for at least the first third of the tasting period. However, these stark results are interestingly not noticeable in the SVM regression model using averaged thickness ratings, as SVM regression works by assigning a specific value to a stimulus. Notably, only the oral SSC is identified as an area that has distributed encoding of thick stimuli, and the model with the 2.5s delay highlights motor and visual areas in addition to the anterior insula. This finding is interesting because of the stark difference between results obtained through univariate GLM and SVM regression.

Table 4.15

Univariate GLM Results of Thickness Ratings

Anatomical location	Co-ordinates x	y	z	$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
Stimulus Onset						
Positive Contrast						
Secondary Visual Area	-36	-86	-8	7.34	0.000	12775
Small Volume Correction at right amygdala [22, 0, -18]						
Amygdala	26	2	-12	3.45	0.003	26
Negative Contrast						
Posterior Cingulate	-18	-40	12	5.49	0.000	8683
Angular Gyrus	46	-64	22	5.35	0.000	526
dlPFC	24	28	36	4.95	0.002	188
Cerebellum	0	-48	-40	4.77	0.033	110
Angular Gyrus	-36	-80	36	4.38	0.003	179
Cerebellum	4	-52	-18	4.07	0.036	108
Small Volume Correction at vmPFC [-2, 44, -4]						
vmPFC	2	48	-10	3.28	0.035	8
2.5 s Delay						
Anatomical location	Co-ordinates x	y	z	$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
Positive Contrast						
Secondary Visual Area	-10	-88	-8	7.02	0.000	15593
Small Volume Correction at right amygdala [22, 0, -18]						
Amygdala	24	0	-12	3.54	0.003	24
Negative Contrast						
Angular Gyrus	46	-62	22	5.56	0.000	11588
Angular Gyrus	-36	-80	36	4.28	0.001	217
Caudate	6	10	-4	4.01	0.000	258
Small Volume Correction at vmPFC [-2, 44, -4]						
vmPFC	4	48	-10	4.02	0.008	28
Small Volume Correction at frontal operculum [56, 12, 8]						
Frontal Operculum	60	8	12	4.17	0.001	65

Table 4.16

SVM Regression Results of Thickness Ratings

Stimulus Onset						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
No supra-threshold voxels survive at whole-brain level.						
Small Volume Correction at oral SSC [62, -10, 20]						
Oral SSC	66	-2	16	3.86	0.001	113
2.5 s Delay						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
Visuomotor Area	26	-70	56	4.23	0.000	2578
Premotor/Supp Motor	8	6	60	4.15	0.073	185
Small Volume Correction at anterior insula [36, 10, 10]						
Anterior Insula	34	2	14	3.17	0.035	5

4.6.3 – Oiliness

As established in Chapter III, oiliness ratings appear to be tightly correlated with the coefficient of sliding friction of orally delivered stimuli, being the psychophysical rating related to how lubricative the oral stimulus is perceived to be. However, as oiliness ratings were not obtained during the scanning sessions themselves, the oiliness ratings from the behavioural pre-testing sessions were collated and the stimulus-specific means were used as the values for the SVR regression. Although this does not address trial-to-trial variability in the internal state of participants, as it uses an average value from a previous session, it is still highly effective at reducing inter-participant variations.

The univariate GLM using the stimulus-specific means of oiliness ratings did not yield any clusters above the cluster-cutting threshold. However, according to the results of the SVR regression on oiliness ratings, the OFC, the oral SSC and the amygdala are all involved in the encoding of the oiliness of oral food stimuli (Fig. 4.10). Notably, the OFC effect appears to be bilateral, with a larger cluster appearing in the left OFC stretching into areas of the vmPFC, although this cluster is not in itself large enough to fulfil our cluster-cutting threshold. Nonetheless, it is apparent that the areas involved in the processing of the oiliness of the stimuli are also areas noted for their processing of textural properties of oral food stimuli. The oral SSC in itself encodes oral somesthetic stimuli (Tamura et al., 2008), whereas the amygdala has also been implicated in oral fat processing (Grabenhorst, Rolls, et al., 2010). The results also expand the role of the OFC, previously known to encode viscosity of oral stimuli as well as fat content, to that of also encoding the CSF as reflected by the oiliness ratings of the oral food stimuli, implicating both the lateral and medial OFC as well as the adjacent vmPFC.

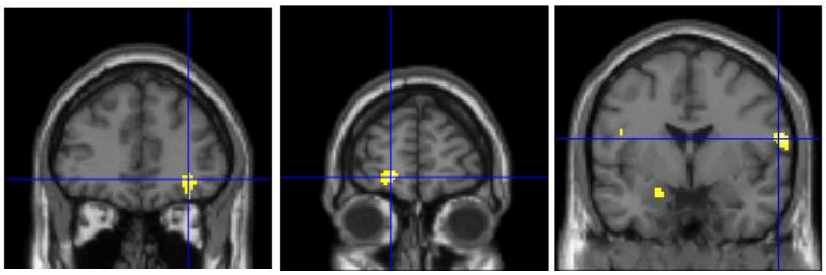

Figure 4.10. *Left* Lateral OFC encoding of oily oral stimuli. *Middle* Medial OFC/vmPFC encoding implicated in oily oral stimuli. *Right* Oral SSC and amygdala processing of oily oral stimuli.

Table 4.17

SVM Regression Results of Oiliness Values

Stimulus Onset						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
No supra-threshold voxels survive at whole-brain level.						
Small Volume Correction at OFC [32, 34, -14]						
OFC	34	40	-8	3.58	0.005	57
Small Volume Correction at oral SSC [62, -10, 20]						
Oral SSC	62	-2	18	3.94	0.006	52
Small Volume Correction at left amygdala [-20, 0, -12]						
Amygdala	-20	-2	-18	3.41	0.020	17
2.5 s Delay						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
Angular Gyrus	-52	-54	36	4.42	0.004	362
Premotor/Supp motor	-32	-2	50	4.36	0.017	265
Small Volume Correction at vmPFC [-2, 44, -4]						
Dorsal ACC	-8	36	-8	3.55	0.022	14

4.6.3.1 – Conjunction of Sliding Friction and Oiliness

Although the previous chapter established the strong relationship between CSF and oiliness, as the oiliness ratings reflect the CSF of the oral food stimuli, how these two variables are linked in the brain is still largely unknown. As oiliness is in itself a psychophysical rating that is provided by participants, whereas CSF is an objective property describing the interaction of the stimulus and its contact surfaces, it would be interesting to explore where in the brain this textural parameter is then processed into an oiliness value.

The conjunction analysis identifies a large shared cluster in the OFC, showing that there is indeed shared processing of stimuli with low CSF stimuli perceived to be oily by participants (Fig. 4.11), which is likely due to the role of the OFC in integrating modalities into a valuation of the oral food stimulus. Interestingly, there is no significant overlap in other structures linked to the somesthetic processing of oral stimuli, such as the midposterior insula or the oral SSC.

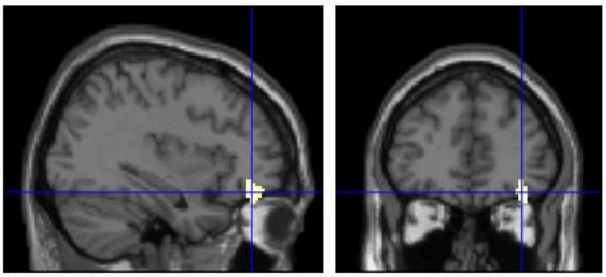

Figure 4.11. Conjunction of CSF and oiliness in the OFC.

Table 4.18

Conjunction of Oiliness and CSF Accuracy Maps

Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	y	z			
OFC	36	42	-18	3.68	0.002	84

4.6.4 – Sweetness

Another psychophysical rating that the participants were asked during scanning was sweetness. As established in the previous chapter, sweetness ratings are closely linked to the sugar concentration of the stimuli. Therefore, the participants' trial-by-trial sweetness rating in essence reflects their subjective perception of the amount of sugar in the stimuli. Due to the strong link between subjective sweetness and reward value (see Chapter III), modelling the neural responses to sweetness would show the sweet taste component contribute to reward value.

No supra-threshold clusters were identified using trial-by-trial sweetness ratings as a parametric modulator for the pump delivery regressor. SVM regression using the averaged values of sweetness ratings for each stimulus highlighted the amygdala as a region that may be involved in sweetness processing, although the location of the cluster was slightly further posterior than the SVC coordinates such that the cluster formed during SVC was very small (Table 4.19).

The lack of strong results is quite surprising, due to the strong effect sweetness has on reward value. One would expect many reward areas to be implicated in the processing of sweetness. However, the design of the study, with only two low-sugar stimuli and the others being high-sugar, does not in itself focus on how sweetness modulation changes reward value. Adding more variation in sugar concentration among the stimuli would possibly have yielded more meaningful results for this psychophysical rating.

Table 4.19

SVM Regression Results of Sweetness Values

Stimulus Onset						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
No supra-threshold voxels survive at whole-brain level.						
Small Volume Correction at left amygdala [-24, 0, -12]						
Amygdala	-18	4	-20	3.23	0.015	2
2.5 s Delay						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
No supra-threshold voxels survive at whole-brain level.						

4.6.5 – Binary Fat Choice

The final psychophysical rating that was modelled in participants was the binary fat rating, where participants are asked to rate if the stimulus they have just tasted contained fat by answering 'yes' or 'no'. As the fatty stimuli were clamped at a constant amount of fat, although the soya fat stimulus contained 30g of fat as opposed to 36g, there is a substantial difference in fat levels between the low-fat and the high-fat stimuli. This rating task, therefore, tests the accuracy of fat detection in participants. Hence, modelling the binary fat choice would highlight areas involved in the judgement of the fat content of stimuli.

A searchlight SVM classifier trained to decode fat choice between yes and no from multi-voxel activity showed high decoding accuracy in the posterior insula and the frontal operculum (Fig. 4.12). At 2.5s after stimulus delivery, decoding accuracy was observed in the anterior insula with an SVC. These results indicate that the insula, especially the posterior insula and the opercular area adjacent to the oral SSC predict if participants will perceive the stimulus as fatty.

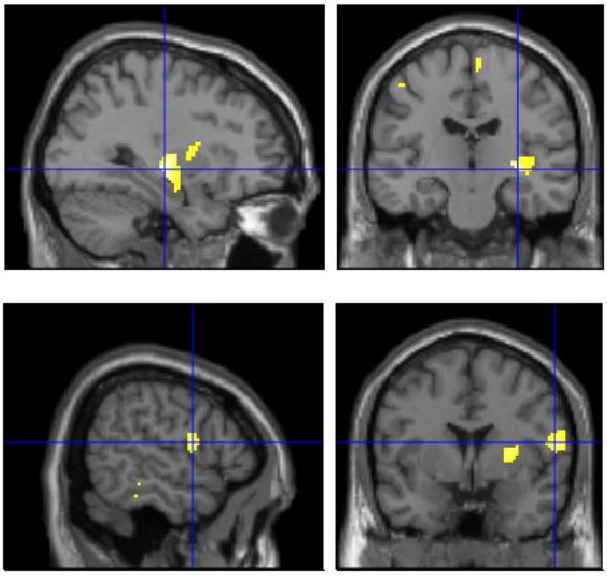

Figure 4.12. *Top* MVPA results of binary fat choice in posterior insula. *Bottom* MVPA results of binary fat choice in frontal operculum/oral SSC.

Table 4.20

SVM Classification Results of Binary Fat Choice

Stimulus Onset						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
Posterior Insula	32	-18	-4	4.15	0.011	513
Small Volume Correction at frontal operculum [56, 12, 8]						
Frontal Operculum	60	4	12	3.54	0.021	11
2.5 s Delay						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
Small Volume Correction at taste insula [36, 10, 10]						
Anterior Insula	34	2	12	3.29	0.017	16

4.6.6 – Fat Choice Probability

Modelling fat choice in a binary way implicated the posterior insula and operculum in fat perception. The results from the previous SVM are informative, although still sparse. As the online ratings were used as parametric modulators, only half of the total trials were used and this likely led to reduced power in the analyses. In order to counter this, averages were obtained of the probability that each stimulus is marked as fatty, which was then termed 'fat choice probability'. In doing so, all the trials, including sweetness and thickness rating trials can be used.

Running an SVM regression using these parameters indicated that the midposterior insula and the oral SSC encode the subjective perception of fat content, regardless of actual fat content (Table 4.21). While using the SVC highlights different parts of this cluster, Figure 4.13 shows the cluster of interest, from which the fact that the decoding cluster is actually large enough to extend from the midposterior insula to the oral SSC. Using the 2.5s delay, one also notes a large area in the SI that extends through to the premotor and supplementary motor cortices. Due to the documented involvement of the oral SSC and the midposterior to posterior insula in the encoding of more somesthetic stimuli independent of taste, the results here further lend support to the idea that fat detection relies heavily on the oral textural parameters of the food item.

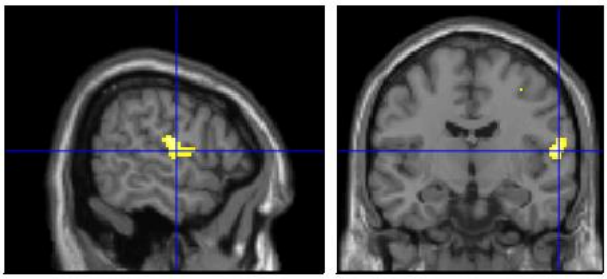

Figure 4.13. SVM regression results showing a cluster stretching from the midposterior insula to the oral SSC.

Table 4.21

SVM Regression Results of Fat Choice Probability Values

Stimulus Onset						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	X	**y**	**z**			
No supra-threshold voxels survive at whole-brain level.						
Small Volume Correction at midposterior insula [48, -8, 12]						
Midposterior Insula	56	-8	12	3.94	0.012	30
Small Volume Correction at oral SSC [62, -10, 20]						
Oral SSC	58	-10	12	4.03	0.002	87
2.5 s Delay						
Anatomical location	**Co-ordinates**			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ **(cluster)**	k_e
	x	**y**	**z**			
Angular Gyrus	-34	-64	32	4.74	0.000	2918
Supramarg Gyrus	-46	-32	42	4.19	0.001	438
Premotor/Supp Motor†	18	2	50	3.85	0.031	232
Fusiform Area	28	-46	-10	3.75	0.011	296

† *This is a large area that stretches across the primary sensory cortex [40, -14, 42]*

4.6.6.1 – Conjunction of Viscosity, Sliding Friction and Fat Choice Probability

The overlap of areas involved in the processing of the fat-related textural parameters, namely viscosity and CSF, and fat choice probability is also interesting. As there is a lack of consensus of the exact mechanism involved in oral fat detection, overlapping areas would imply that these textural parameters are indeed used to provide information on oral fat content.

The conjunction analysis shows a small section of the midposterior insula encoding both the textural parameters as well as how likely the participant reports the detection of fat in the stimulus. This result, taken together with the documented function of the midposterior insula in the processing of somesthetic information of the oral cavity (de Araujo & Rolls, 2004), further supports the theory of oral fat detection occurring through detecting the changes in oral textural parameters. Most interestingly, this also implies that the midposterior insula is the structure from which the participants draw this information and unifies it into a sensation of fattiness.

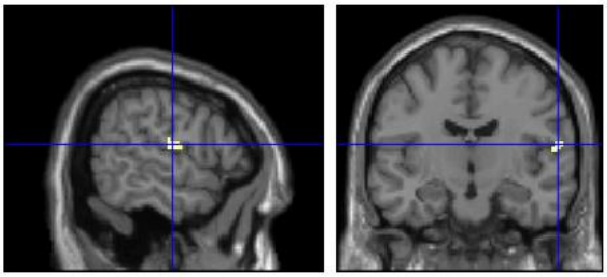

Figure 4.14. Conjunction of viscosity, CSF and fat choice probability in the midposterior insula.

Table 4.22

Conjunction of Viscosity, CSF and Fat Choice Probability Accuracy Maps

Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	y	z			
No supra-threshold voxels survive at whole-brain level.						
Small Volume Correction at oral SSC [62, -10, 20]						
Oral SSC	60	-12	14	3.96	0.010	39

4.7 – Fat Detection in Control Stimuli

The previous chapter highlighted the phenomenon wherein the participant population was divided on the reported fat content of the CMC and that of the protein stimulus. This manifested in a bimodal distribution where a proportion of participants correctly reported these control stimuli as lacking in fat, whereas many still reported them as fatty. The neuroimaging data may help shed light on this phenomenon, to explore the differences in fat detection among participants. To that end, the fat choice probability SVM regression maps were used in a second-level analysis, where each participant's fat choice probability for the protein stimulus and the CMC stimulus were entered as covariates respectively. The resultant maps then show the structures whose variance in decoding accuracy is explained by the participants' fat choice probability for the specific stimulus, thereby showing the difference in processing related to the perceived fat content of the stimulus.

4.7.1 – Protein

Performing the multiple regression analysis with the fat choice probability of the protein stimulus as a subjective covariate yields the results in Table 4.23. There is a large cluster stretching from the left amygdala through to the medial OFC, in addition to a cluster in the lateral OFC (Fig. 4.15). Decoding accuracy in these structures is positively correlated with the participants mistakenly reporting the protein stimulus as fatty, thereby implying that there is a distinction in encoding here between participants who are susceptible to fat replacement through protein and those able to accurately report it as lacking in fat. This indicates that these structures contribute to the overall decision of reporting fat content, specifically that the mechanism for fat replacement through protein requires some additional inputs from the amygdala and the medial and lateral OFC.

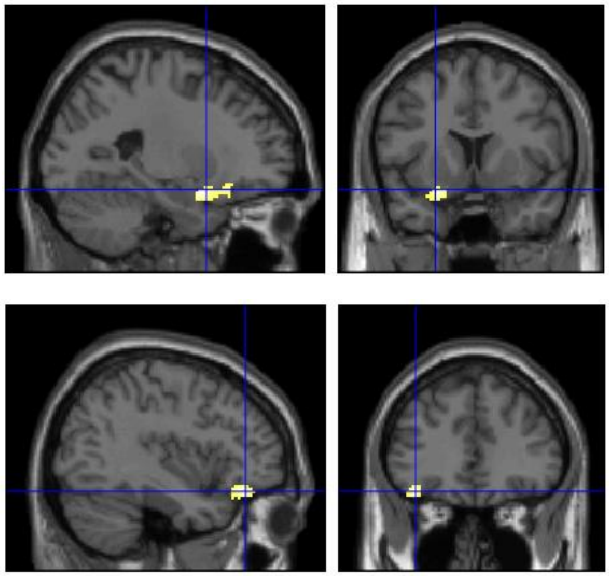

Figure 4.15. *Top* Peak of the cluster stretching between the amygdala to the medial OFC. *Bottom* Peak of the cluster in the lateral OFC.

Table 4.23

Multiple Regression Results with Fat Choice Probability of Protein Stimulus

Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	y	z			
Positive Contrast						
Amygdala/OFC	-20	6	-18	3.84	0.019	243
Small Volume Correction at left OFC [-32, 34, -18]						
OFC	-38	36	-18	3.64	0.003	70
Negative Contrast						
No supra-threshold voxels survive at whole-brain level.						

4.7.2 – CMC

Multiple regression was also performed on the fat choice probability accuracy maps, using the fat choice probability of the CMC stimulus as covariates for each participant. Table 4.24 lists the only result that surpasses the threshold for the positive contrast, a scattered cluster in the vmPFC identified using SVC (Fig.4.16). This implies that the vmPFC, as well as the pregenual cingulate, holds stimulus-specific representations that are more different for participants who are susceptible to mistaking the CMC stimulus for fat. Interestingly, while not strong enough to pass whole-brain correction, the negative contrast in Figure 4.16 highlights an area bordering the superior posterior insula and the oral SSC, implying that participants who successfully report the CMC stimulus as lacking in fat may potentially have greater levels of population encoding in these structures.

Taken together, both of these results indicate the involvement of the vmPFC and posterior insula as areas involved in fat processing. As the vmPFC is known to maintain reward value signals (Kahnt et al., 2011), it may be that the vmPFC misattributes the CMC stimulus as fatty due to the reward value assigned to it. Interestingly, the role of the area spanning the posterior insula and the oral SSC in enhancing the accurate rejection of the CMC stimulus is unexpected, as the CMC stimulus was designed to mimic the high viscosity and low CSF of fat. However, the increase in viscosity and reduction in CSF is disproportionate compared to actual fats, such that this discrepancy in the textural components may indeed help some participants accurately rate the CMC stimulus as lacking in fat.

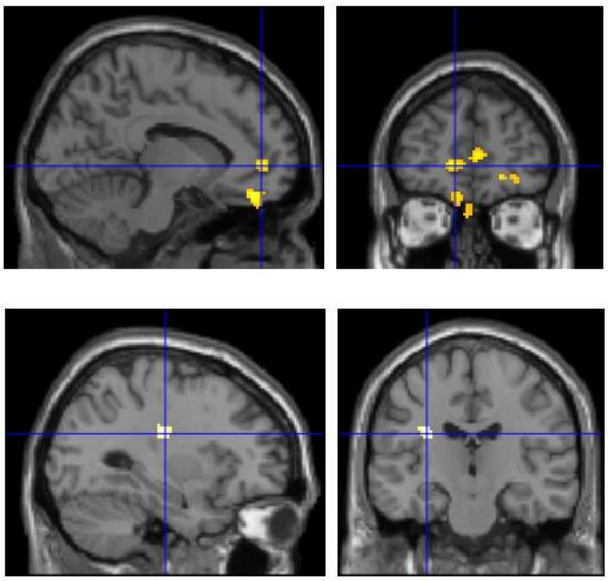

Figure 4.16. *Top* Sparse clusters in the vmPFC and pregenual cingulate correlated with high fat choice probability of CMC. *Bottom* Small cluster in the posterior insula/oral SSC border negatively correlated with fat choice probability of CMC.

Table 4.24

Multiple Regression Results with Fat Choice Probability of CMC Stimulus

Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	y	z			
Positive Contrast						
No supra-threshold voxels survive at whole-brain level.						
Small Volume Correction at vmPFC [-2, 44, -4]						
vmPFC	-10	48	0	3.52	0.019	18

4.8 – Connectivity Analyses

The results of this chapter have highlighted neural structures that respond to specific stimuli or parameters in the design, such as the high-fat stimuli or the coefficient of sliding friction of the stimuli tasted. However, this does not in itself show how the structures interact with each other. Therefore, a means of testing connectivity between structures is required.

The psychophysiological interaction (PPI) analysis provides a tool for connectivity analysis with fMRI data (Friston et al., 1997), as it highlights the task-dependent correlation of BOLD responses in different structures. In essence, PPI functions by selecting a seed region from which to base the analysis and examining the fluctuation in correlation of the BOLD activity of said region with the rest of the brain as a function of a specific task or stimulus contrast. To that end, a GLM is conducted with the task or contrast time course (psychological) and the time course of the BOLD response of the seed region (physiological) as covariates of no interest, in addition to the demeaned scalar product of the two time courses (interaction). In doing so, the analysis accounts for inherent correlations among regions that share neuro-modulatory influences and inputs as well as regions that are simultaneously involved in a task, focusing instead on the regions where the correlation of the neuro-modulation increases with the seed region specifically during the task or stimulus of interest. Therefore, structures that are highlighted in the positive contrast of the PPI analysis have increased correlated activations with the seed region as a function of the specific task or stimulus contrast, implying an increase in coupling, whereas the negative contrast highlights structures with task-dependent reduced correlation in BOLD signal, implying de-coupling. However, while the region from which the initial modulation is extracted is termed the seed region, PPI analysis does not show the directionality of the coupling, such that the seed region may be receiving inputs or outputs from the coupled region during the task.

PPI analyses in this thesis extracted BOLD data from the peak voxel, identified from the previous univariate GLM and MVPA, for the modelling, and for each analysis the stimulus contrasts are specified.

4.8.1 – Stimulus-Rinse

The stimulus-rinse contrast yielded strong results in both the univariate GLM and the SVM regression analyses, as every trial had a stimulus onset and a rinse onset. Therefore,

it provided the best starting point for PPI analyses, as it would indicate increased coupling between structures during real-time consumption of oral food stimuli, as opposed to having a tasteless isotonic rinse in the oral cavity. Therefore, the stimulus onset was used as the positive task contrast, whereas the rinse was used as the negative task contrast.

PPI analysis on the stimulus-rinse contrast with the anterior insula as the seed region indicates increased coupling between the insula and the oral SSC during all stimulus consumption (Fig. 4.17). There also appears to be increased coupling between the insula and the hypothalamus. Using the hypothalamus peak as the seed region, on the other hand, highlighted increased coupling between the hypothalamus and the oral SSC as well as both the anterior and posterior insular cortices (Fig. 4.17). As this contrast is a general comparison between the consumption of any caloric stimulus and a tasteless, non-caloric, isotonic rinse, in addition to the known role of the hypothalamus as a metabolic regulatory structure, it is likely that the hypothalamus receives inputs from these assorted structures regarding the various taste and textural properties of the oral food stimuli for the purposes of satiety signalling.

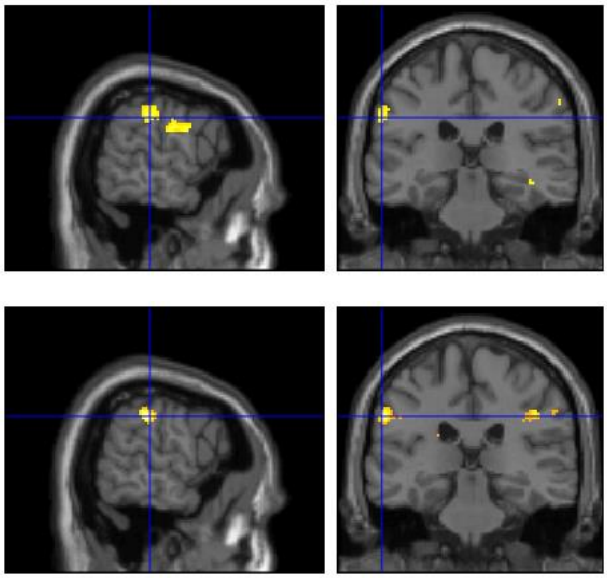

Figure 4.17. *Top* Increased coupling between the insula and oral SSC during stimulus consumption. *Bottom* Increased coupling between the hypothalamus and the bilateral oral SSC during stimulus consumption.

Table 4.25

PPI Results with Stimulus vs Rinse Contrast

Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	y	z			
Insula Seed at [30, 24, -4]						
Positive Contrast						
Oral SSC	-60	-28	32	3.79	0.040	99
Small Volume Correction at left ventral striatum [-12, 2, 0]						
Hypothalamus	-10	0	0	3.82	0.014	19
Negative Contrast						
Fusiform Area	-28	-48	-16	4.91	0.000	1054
Angular Gyrus	-26	-78	32	4.37	0.000	500
Hypothalamus Seed at [12, 0, 0]						
Positive Contrast						
Oral SSC	-60	-28	32	4.90	0.011	117
Anterior Insula	-22	24	12	4.47	0.036	92
Posterior Insula/Oral SSC	42	-30	38	4.25	0.002	155
Negative Contrast						
Premotor/Supp Motor	-44	8	56	4.11	0.001	190

4.8.2 – Coefficient of Sliding Friction

In addition to the stimulus-rinse contrast, PPI analysis was also conducted on CSF, as oral CSF processing in humans is still largely unexplored. In order to do so, the stimuli were separated into high-CSF and low-CSF stimuli using a median split of the CSF values. The positive contrast for this model was the low-CSF stimuli, whereas the negative was the high-CSF stimuli, due to the negative correlation between CSF and fat content.

The PPI analysis of the high-CSF versus low-CSF contrast using an OFC seed region shows increased coupling with the bilateral oral SSC/posterior insula region (Fig. 4.18). However, this result is not particularly strong. The SVC coordinate used was that of the fattiness ratings in Grabenhorst and Rolls (2014), as the role of CSF in informing the OFC about the fat content of oral stimuli is of interest, as opposed to specifically the pleasantness of the stimuli tasted. It is likely that the weak result arose from the median split used for the PPI analysis resulting in a lower power, as only half of the trials are used for each contrast as opposed to all trials in the stimulus-rinse contrast. Furthermore, the CSF values of the stimuli are distributed along a continuum, such that creating a binary distinction may have removed meaningful granular distinctions. Nevertheless, it does appear that the OFC and oral SSC experience increased coupling during the consumption of low-CSF food stimuli. This echoes previous findings showing increased coupling in these structures during the consumption of pleasant fatty stimuli (Grabenhorst & Rolls, 2014), implying that the component of reward value that arises from fat due to its textural properties, which is processed in the oral SSC, is forwarded to the OFC in response to the presence of low-CSF oral stimuli.

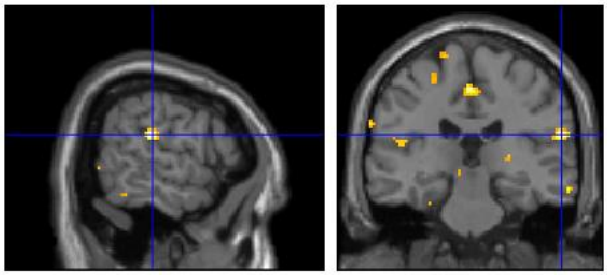

Figure 4.18. Increased coupling between OFC and Oral SSC during consumption of low-CSF stimuli. Map displayed at voxel-level $p<.005$ for display purposes.

Table 4.26

PPI Results with CSF Contrast

Anatomical location	Co-ordinates			$Z_{\equiv}$	$p_{FWE\text{-}corr}$ (cluster)	k_e
	x	y	z			
OFC Seed at [32, 46, -14]						
Positive Contrast						
No supra-threshold voxels survive at whole-brain level.						
Small Volume Correction at oral SSC [66, -22, 28]						
Oral SSC	62	-26	20	4.43	0.020	13
Negative Contrast						
No supra-threshold voxels survive at whole-brain level.						

4.9 – Summary

This chapter has explored the specific neural signals correlated with well-defined objective and subjective parameters of the food-stimulus set. The first contrast, a general pump-rinse contrast, served as a baseline test and identified areas that are putatively involved in the processing of oral food stimuli and reward valuation, with notably strong effects in all parts of the insula, oral SSC, amygdala, hypothalamus, ventral striatum and vmPFC. The analyses were further refined to determine signals related to reward value following the same progression of determinant modelling laid out in Chapter III, starting from objective measures such as the caloric load and macronutrient contents of the stimuli to the subjective ratings provided by participants during scanning. In doing so, the cortical and limbic structures that are involved in taste, texture and reward processing were highlighted. The structures that are repeatedly implicated are the OFC, the oral SSC, the vmPFC, the amygdala and both the granular and agranular insular cortices, with a few analyses also showing effects in the hypothalamus.

Areas such as the oral SSC and the dysgranular and granular insula have both been shown to respond to somesthetic stimulation in the oral cavity (de Araujo & Rolls, 2004; Manger et al., 1995; Merzenich et al., 1978). These findings were replicated and expanded on in this project through results identifying both the oral SSC and midposterior insula in the processing of textural parameters of the oral food stimuli (viscosity and CSF) and their corresponding subjective ratings (thickness and oiliness). Subjective ratings of fat content also appear to be derived from information contained in these structures, as seen from the fat choice probability SVM regression maps. Hence, it is likely that the mechanism behind fat detection in oral food stimuli occurs through the processing of

their textural parameters in the mid-to-posterior insula and oral SSC, which is subsequently relayed to the OFC for valuation. Indeed, this is reflected in the increased OFC-granular insula/oral SSC coupling during the consumption of foods with low CSF. This result expands previous findings demonstrating increased coupling between the OFC and the oral SSC during the consumption of oral fat (Grabenhorst & Rolls, 2014) by identifying CSF as the specific oral texture parameter involved in the processing of oral fat.

Congruent with these results, the OFC has also been prominent in the processing of textural parameters such as viscosity and CSF, as evidenced by the conjunction of these effects in the OFC. However, the OFC is also unique in that it also encodes subjective reward in addition to these parameters, which implies that sensory information from the various sensory modalities converge and are integrated in the OFC. In addition, the subjective ratings of oral textural properties (oiliness and thickness) are also encoded in the OFC, with oiliness encoding occurring bilaterally. These results suggest that the OFC bilaterally integrates reward value from the various sensory modalities, as previous studies have also reported activations to oral fat stimuli in the left hemisphere (de Araujo & Rolls, 2004). In line with previous research in the role of the OFC in integrating reward components into an overall reward value in terms of olfactory reward identity (Howard et al., 2015), nutrient component from visual stimuli (Suzuki et al., 2017) and value-specific oral fat stimuli (Grabenhorst, Rolls, et al., 2010), the OFC is identified in this project as an integratory hub of sensory information which is then formed into a stimulus-specific reward value. This idea is indeed reinforced by the latency in value encoding in the vmPFC (only with a 2.5s delay). Through the well-documented gradation of value encoding in the OFC, in that it laterally encodes specific properties of reward and encodes reward value independent of identity in a medial region near the vmPFC (Howard et al., 2015; Kahnt et al., 2011; Suzuki et al., 2017), our results imply that the OFC integrates value signals from the textural properties of oral food stimuli before forwarding them to the vmPFC for further evaluation of the food item, where a decision regarding further consumption is processed.

Behavioural data from the previous chapter highlighted an interesting phenomenon whereby a difference in participants' ability to accurately report the control stimuli as lacking in fat was found. The differences in neural encoding explained by

participants' ability to distinguish either the protein stimulus or the CMC stimulus from actual fat have also been explored in this chapter, implicating regions of the brain, such as the connecting structure between the amygdala and the medial OFC in the protein stimulus and the vmPFC in the CMC stimulus. Furthermore, there is a weak but notable difference in encoding in the posterior insula-oral SSC region that predicts participants' ability to report the CMC stimulus as fat-lacking, implying that the ability for participants to reject the CMC stimulus may be due to more finely tuned textural perception rather than an a chemosensory phenomenon. This further implies that a specific fat-taster status in humans, such as 'oleogustus' (Running et al., 2015), is an unlikely mechanism of fat taste and that participants who are able to distinguish fatty stimuli from non-fatty stimuli are more sensitive to the textural discrepancies, such as the ratio of the increase in viscosity to the decrease in CSF in the oral cavity.

Chapter V – Discussion

5.1 – Overview of Findings

In this section, we describe the findings from the behavioural and the neuroimaging components of the study. We explore the main findings of each component of the study, integrate across study components and consider how they expand current understanding of nutrient detection and reward valuation of foods. The findings are presented in the context of the following three broad hypotheses, as laid out in the introduction:

- The nutrient content of oral food stimuli is sensed through a combination of taste and textural properties.
- The economic value of each stimulus is determined by the nutrient content, which in turn is detected by the psychophysical properties of the stimulus.
- Distinct neural signals reflect specific nutrients from sensory food properties and their economic valuation.

The behavioural component of this project extensively addressed the first two hypotheses. Psychophysical ratings were correlated strongly with the macronutrient compositions of oral food stimuli. Specifically, the sugar content was predictive of the sweetness ratings, whereas the fat content was reflected in the thickness and oiliness ratings. The high-protein stimulus was not found to be identifiably distinct from other stimuli in the psychophysical ratings explored, even from subjective ratings of protein content. Thickness and oiliness ratings also correlated with the textural parameters of the stimuli, namely viscosity and coefficient of sliding friction (CSF). Subjective reports of fat content of the control stimuli indicated that participants were divided into those who could accurately report control stimuli as fat-lacking and those who confused them for fatty stimuli.

In addition, the subjective value for the stimulus set, as obtained through the Becker-DeGroot-Marschak (BDM) auction task (Becker et al., 1964), was not found to be satisfactorily predicted through the pure caloric load of the stimuli but better modelled by their nutrient compositions. The model with the textural parameters of the stimuli slightly outperforms the pure macronutrient model, although the best predictor of subjective value is the model using the trial-by-trial psychophysical ratings as regressors. This implies that nutrient content is detected through the textural parameters, which

participants express as the corresponding psychophysical ratings. The ecological validity of the BDM task and the nutrient model is also supported by the finding that the beta weights of the fat regressor in this model are correlated positively with the percentage of fat that participants consume during an ad-libitum free-eating experiment under life-like conditions.

Meanwhile, the findings from the fMRI scanning task address the third hypothesis that there are distinct patterns associated with the perception of different nutrients. Specifically, the processing of the textural parameters of fat occurs in the oral somatosensory cortex (SSC), the midposterior and posterior insula, as well as the lateral orbitofrontal cortex (OFC). These effects occurred in response to both the viscosity and the CSF of the oral stimuli presented. Crucially, this study is the first report of neural population encoding of the CSF of real-time food stimuli in humans. A conjunction analysis of these textural parameters with the fat rating maps highlights the midposterior insula/oral SSC area as the unique structure in which all three texture-sensing processes occur, whereas a conjunction of the effects related to textural parameters and the BDM values highlight the lateral OFC area. In addition, functional coupling between the OFC and oral SSC increased during the consumption of oral stimuli low in CSF.

This chapter will place these findings in the context of the wider literature in the field, describing how the study has advanced the field of oral fat detection and reward valuation. The specific phenomena of nutrient detection will first be addressed, after which we will discuss how these taste and textural components are integrated into a whole reward value. Subsequently, the main brain structures of interest will be explored in turn, focusing particularly on the structures in which stronger conclusions can be obtained through their involvement in a number of the phenomena explored in this thesis. Further, clinical and industrial applications of the results of this project will be discussed, followed by an examination of the limitations of this study and how it may influence future work in the field.

5.2 – Nutrient content sensing

In order to test the first hypothesis on how nutrients are sensed during food consumption, we explore the psychophysical ratings as well as the neuroimaging data during oral food stimulus receipt. The main nutrients in the stimulus set used in this

project were fat and sugar, with a greater emphasis on fat. It is important to explore the two mechanisms by which these nutrients are sensed, namely taste and texture.

5.2.1 – Sugar and Sweetness

The sensory modality of taste, which occurs through the activation of chemoreceptors on the taste buds of the tongue, detects the five main tastes of sweet, salty, sour, bitter and umami (Ikeda, 2002; McCabe & Rolls, 2007). However, within the nutrients of interest in this project, the only pertinent taste of the main five tastes is sweetness, which is associated with the presence of either monosaccharides or disaccharides in the oral cavity through their activation of taste 1 receptors family members 1 and 2 (T1R1 and T1R2; Chandrashekar, Hoon, Ryba, & Zuker, 2006; A. A. Lee & Owyang, 2017). Therefore, the sweetness of oral food stimuli is an indicator of the simple sugar content of the food item.

The findings of this study implicate sweetness and the sugar content in the determination of the subjective value of oral food stimuli, as evidenced by the behavioural pre-testing results. In all models involving either sugar concentration or sweetness ratings, sugar-related regressors are consistently shown to be strong positive predictors of the BDM values, indicating that in most participants sugar concentration or the resultant sensation of sweetness does contribute significantly to subjective value. This is in line with the well-established role sugar has in literature as an appetitive stimulus across species (Ahmed, Guillem, & Vandaele, 2013; A. A. Lee & Owyang, 2017; Skorupa et al., 2008; Stice et al., 2013; Wolf et al., 2008). It is of interest that this appetitive effect of sugar is more pronounced in infants, as noted in both rodents and humans (Desor et al., 1973; Wilmouth & Spear, 2009), indicating some level of innateness of sugar preference across species which reduces with age.

Another notable finding from the behavioural pre-testing session is that the addition of fat appears to increase perceived sweetness, as tested through a two-way analysis of variance (ANOVA). Both the high-fat low-sugar (HFLS) and high-fat high-sugar (HFHS) stimuli were perceived to be sweeter than their low-fat counterparts, despite having the exact same levels of sugar concentration (10g/300ml for low-sugar stimuli and 20g/300ml for high-sugar stimuli). This slight effect of the addition of fat enhancing sugar perception, as well as the positive interaction effect between fat and sugar on the sweetness ratings, may be due to a host of factors. One possible mechanism for this effect

is the higher viscosity of the high-fat stimuli leading to reduced mobilisation of dissolved sugar, such that the sugars in the stimuli are diluted more slowly leading to a longer-lasting sensation of sweetness. However, this mechanism would also imply that the high-viscosity CMC stimulus would be seen as higher in sweetness than the low-fat high-sugar (LFHS) stimulus, which was not observed from the rating data. Another possibility is that the current Western diet frequently has fatty and sugary foods presented together, to the extent that the combination of foods rich in both carbohydrates and fat often have greater subjective values than their isocaloric high-carbohydrate or high-fat counterparts (DiFeliceantonio et al., 2018). Therefore, the previously established association between fat and sugar may have contributed to a top-down enhancement of the perception of sweetness. As increased salt content is known to increase sweetness perception, putatively due to the presence of sodium-glucose co-transporter 1 (SGLT1) and ATP-gated potassium channels in T1R3 receptors on the taste buds (Yee, Sukumaran, Kotha, Gilbertson, & Margolskee, 2011), it is possible that the presence of fat may likewise contribute to a currently unknown glucose-sensing mechanism in the oral cavity, thereby explaining the increased sweetness ratings of high-fat stimuli. This phenomenon could be explored further through the use of more levels of sugar concentrations.

From the neuroimaging results, neither sugar content nor sweetness ratings showed meaningful contrasts from the univariate GLMs, although both showed some results using MVPA. This implies more population-based neural encoding of sugar content and the resultant sweetness. MVPA results indicate decoding accuracy in the bilateral mid-insular cortex, which is unexpected due to the differentiation of roles in the insular cortex, with the taste cortex being localised to the agranular anterior insula whereas the dysgranular and granular regions of the insula were expected to encode textural properties, as observed in both humans and non-human primates (G. K. W. Frank et al., 2008; Kadohisa, Rolls, et al., 2005; Kringelbach, de Araujo, & Rolls, 2004; E. T. Rolls, 2011; Stice et al., 2013; Verhagen et al., 2004; Yaxley et al., 1990). However, the stimulus set used in this project does not focus strongly on the sugar content, with the two-low-sugar stimuli being clamped at 10g/300ml and the high-sugar stimuli being clamped at 20g/300ml. In addition to the under-representation of low-sugar stimuli in the experimental stimulus set, the high-sugar stimuli were also highly varied in fat content, protein content and textural parameters, owing to the addition of only high-sugar varieties of the control stimuli. This, therefore, leads to greater difficulty in teasing apart

effects due to the various factors being controlled for and differences arising purely due to different sugar levels.

While the expected cortical structures did not display strong results in either the univariate GLM analysis or the MVPA, a small cluster in the amygdala was found to be encode the subjective sweetness perceptions of the oral food stimuli. Although the location was slightly further posterior than expected, the cluster is still identifiable. This is in line with the known role of limbic system structures such as the amygdala in the encoding and processing of taste-related primary rewards (Alonso-Alonso et al., 2015; Avery et al., 2020; O'Doherty et al., 2001; G. J. Wang et al., 2009). The distinction between decoding accuracies from the objective parameter (sugar content) and that of the subjective parameter (sweetness) is interesting, as the amygdala is only highlighted when training the SVM regression decoder on the stimulus-specific sweetness ratings. Although, as established in the behavioural pre-testing modelling of subjective value, online subjective ratings are better determinants of subjective value as they account for the trial-by-trial variations in intra-participant noise such as attention or amount of saliva already present in the oral cavity, the SVM regression decoder was trained on the stimulus-specific means of sweetness ratings instead of online ratings. Therefore, the differences between the objective sugar content and subjective sweetness ratings should be due to inter-participant differences, implying that the amygdala is involved in how participants derive sweetness ratings from sugar content, more so than the sugar content itself.

5.2.2 – Fat and Texture

The mechanisms responsible for fat detection are widely disputed, with some authors claiming that fat is a sixth primary taste, implying that its detection occurs through a chemosensory mechanism (B. V. Kulkarni & Mattes, 2014; Mattes, 2010; Running et al., 2015). However, lingual lipase in the human oral cavity is unlikely to be at a sufficiently high level to break down triglycerides into fatty acids during mastication. Furthermore, various studies into the textural properties of fat have shown fat-responsive neurons to be responsive to viscosity (Kadohisa, Rolls, et al., 2005) and CSF (E. T. Rolls et al., 2018), with free fatty acids actually eliciting neural responses more similar to non-fatty, low-viscosity stimuli. In line with these findings, this project found that, unlike sugar and sweetness, fat content does not appear to be sensed through taste-related means. Fat

detection is explicitly tested during the task through the binary fat rating. Through this measure, participants report that the high-fat stimuli do indeed contain fat. However, the high-viscosity carboxymethyl cellulose (CMC) stimulus is also seen as fatty by a proportion of the participants. This indicates that these participants who reliably mark the CMC stimulus as fatty rely on its textural properties in order to form this judgement, as CMC is a tasteless non-caloric food thickener. When excluding the CMC stimulus, there is a tight correlation between perceived thickness and the log of the viscosity, in addition to another strong negative correlation between the coefficient of sliding friction (CSF) and oiliness ratings. However, the strength of this correlation is reduced when the CMC stimulus is included, implying that there is a detectable discrepancy between the increased viscosity and reduced CSF, which may have contributed to participants reporting the CMC stimulus as non-fatty.

The CMC stimulus was not the only stimulus mistaken for fat-containing, as a proportion of participants also reported the protein stimulus as fatty, in addition to having an increased subjective value than the LFHS stimulus. As the textural parameters of the high-protein stimulus are much closer to that of the low-fat stimuli than the high-fat stimuli, this implies that some participants do not use the textural properties of the oral stimuli to judge fat content. It is possible that the additional caloric load from the protein content contributed to the decision, indicating that there is a population of participants who are unable to distinguish between added protein and added fat. Another possible mechanism is the post-ingestive effect of the stimulus, leading participants to associate the cue and the stimulus with the increased caloric load, despite not being able to sense the increased protein content at the point of ingestion. This echoes findings in increased preference towards higher-calorie and taste-neutral stimuli after repeated exposure (de Araujo et al., 2013). In order to explore this effect further, especially with respect to individual ability to detect protein orally, a potential experiment could involve the use of a tasteless caloric addition such as maltodextrin and unflavoured protein, such that any differences would be due to the type of macronutrient type rather than simply increased caloric load.

The contribution of textural properties to fat detection is also strongly supported by the neuroimaging findings of this project. SVM regression analysis trained on the viscosity and CSF values of the stimuli show neural population-level encoding in the mid

insula, posterior insula, oral SSC and the OFC. These regions have been shown in various primate and human studies to encode textural properties, separately from taste properties (de Araujo & Rolls, 2004; Grabenhorst & Rolls, 2014; Kadohisa, Rolls, et al., 2005; E. T. Rolls, 2011; Yaxley et al., 1990). These regions also appear to encode fat content, with an SVM classifier trained on fat content showing high decoding accuracy in a cluster stretching from the midposterior insula through to the oral SSC. Furthermore, an SVM classifier trained on subjective trial-by-trial subjective fat perception also implicates the oral SSC, and the midposterior insula. Taken together with the results SVM regression analysis of the fat choice probabilities of each stimulus also highlighting the oral SSC and midposterior insula, it is likely that participants draw on the textural properties encoded in these structures in order to detect the fat content of the oral food stimuli. The three-way conjunction of the viscosity, CSF and fat choice probability maps on the midposterior insula/oral SSC area strongly supports the importance of these textural properties in fat detection, as it highlights this particular juncture of the midposterior insula and oral SSC as the integration of the textural parameters into information about the fat content of the stimulus.

In addition to the neural structures known to be sensitive to somesthetic input, a large cluster of high decoding accuracy was found in the OFC in response to oral stimulus viscosity and CSF. A substantial argument against the idea that fat detection in humans occurs through textural mechanisms is that, while neurons in these areas and the OFC encode the viscosity of oral food stimuli and fat content, there are neurons wherein the encoding of fat is independent of viscosity (Kadohisa, Rolls, et al., 2005; Verhagen et al., 2003). Rolls et al. (2018) identified these neurons in the OFC again and identified their responses to the CSF of oral food stimuli, independent of viscosity. The population encoding of CSF found in the OFC in this project reinforces this finding, suggesting the presence of CSF-sensitive neurons in the OFC. The large overlap of the viscosity and CSF maps indicate that these various structures process these textural parameters. However, one may also attribute this overlap to the strong collinearity between viscosity and CSF, as the increased viscosity from fat is accompanied by a decrease in CSF. It is crucial to note, nevertheless, that the peaks of the CSF and the viscosity maps are distinct, indicating that these parameters are processed differently in the same areas, possibly owing to the disproportionate increase in viscosity and reduction in CSF from the CMC stimulus. Furthermore, while the MVPA results do share large overlapping structures, one should

also note the vast distinctions in the univariate GLM results, where large clusters appear on both the positive and negative CSF contrasts in the bilateral SI, whereas a large cluster is found on the left SI only in the positive viscosity contrast. The univariate GLM results inform us further by indicating that CSF encoding is more spatially distributed in the SI than viscosity encoding. Crucially, a small cluster was apparent in the OFC in the negative CSF contrast which was not found in the viscosity contrast, implying that the OFC has neurons responsive to both viscosity and CSF but has more neurons in the population tuned to decreasing CSF in the oral cavity. Therefore, it is reasonable to claim that the oral SSC, midposterior insula and the OFC all encode the CSF of oral food stimuli, which is then used to inform the fat content of the oral stimulus.

5.3 – Integration of Reward Value

The mechanisms by which the reward value of food is derived from oral sensory mechanics, be they chemosensory or somesthetic, is also an important fundamental biological question, as it then informs how certain foods, especially those high in fat and sugar, are perceived as more rewarding than others. It is therefore key that the mechanism behind the derivation and integration of reward value be investigated in this thesis. The second hypothesis of this project predicted that reward value is derived from specific nutrient contents of the oral food stimuli, which was explored thoroughly through modelling and model comparison techniques in Chapter III, where the various possible mechanisms behind the determination of subjective reward value are fitted into general linear models (GLMs).

One of the oft-cited obesogenic features of the typical Western diet is its high composition of calorie-dense foods. Indeed, overfeeding on calorie-dense foods is often attributed as one of the main causes of the increasing levels of obesity and overweight in the global population (Drewnowski & Darmon, 2005; Karl & Roberts, 2014). Calorie-dense foods are known to be rewarding to humans from early infancy (Gibson & Wardle, 2003) to the extent that visual stimuli of high-calorie foods are more distracting than visual stimuli of low-calorie foods (Cunningham & Egeth, 2018). Taken together, these results imply that calorie density is an attractive quality, to which the reward and reinforcement learning neural systems should be finely tuned. However, although the caloric load of the oral food stimuli did have some bearing on the overall reward value, as seen in Chapter III, the calorie model was not the best model of subjective reward.

Compared to all the other models, the mean Akaike Information Criterion (AIC) of the calorie model was the highest, indicating the poorest fit of the model, suggesting that subjective value is derived from components that are more nuanced than pure calorie content. This is especially of interest as these results indicate that reward value is tuned to something more specific than caloric density of foods. Indeed, the fMRI results presented in Chapter IV strongly echo this notion, with caloric load being associated with decoding in few areas. Due to the role of the hypothalamus in homeostatic regulation (Berthoud, Münzberg, & Morrison, 2017; Farooqi, 2014; Petrovich, 2013; Sternson & Eiselt, 2017), one would expect the hypothalamus to signal the caloric load of the food stimulus, although the lack of encoding in more cortical reward structures implies that caloric load ceases to be a crucial factor. The linear relationship between pure caloric density and rated liking and pleasantness has been documented to collapse above a certain cut-off point of energy density, estimated to be 1.5kcal/g (Brunstrom et al., 2018). It is possible that, after this threshold, caloric requirements are no longer deemed overly vital, such that one is able to be more lenient with one's food choices.

The second hypothesis of this project was aimed at the extent to which nutrient contents contributed to subjective reward values of oral food stimuli. To test this hypothesis, we used GLMs on the BDM results with macronutrient levels as regressors, which appear to be more predictive than GLMs using only pure caloric load. Out of the two models tested, the GLM with only fat and sugar as regressors had significantly lower AICs, indicating that they had a better trade-off between the number of parameters and the fit of the model. This is likely due to the low variation in protein in all stimuli, apart from the very high protein content in the high-protein control stimulus, whereas fat and sugar contents were more varied in the stimulus set. The strength of the model using fat and sugar content as regressors implies that, within the stimulus set tested, sugar and fat content did indeed determine reward value, implying that reward valuation is tuned more specifically to the nutrient content of the oral food stimulus. The fMRI results from Chapter IV also indicate that both fat and sugar engage neural structures more strongly than protein in our stimulus set, with fat decoding being observed in the amygdala, midposterior insula and oral SSC. Furthermore, an analysis with a 2.5s delay showed fat decoding in the vmPFC/pregenual cingulate, which is known to encode task-oriented subjective value (Chib et al., 2009; Kahnt et al., 2011; S. Lee, Yu, Lerman, & Kable, 2021; Strait et al., 2014). Therefore, this neural representation of valuation implies that nutrient

content, especially fat content in this stimulus set, contributes greatly to subjective reward, which was notably missing in the model based on pure calorie density. These findings are consistent with the fact that various nutrients fulfil different and highly specific roles within the body, with the most notable examples being sugar selectiveness (Søberg et al., 2017; Stice et al., 2013) and protein leveraging (Raubenheimer & Simpson, 2019; Simpson & Raubenheimer, 2005; Sørensen et al., 2008). Fat-selective reward value and its neural correlates have also been extensively documented (Grabenhorst, Rolls, et al., 2010; Hoebel, Avena, Bocarsly, & Rada, 2009), with its interaction with carbohydrates in specific being shown to induce greater reward valuation than isocaloric mono-nutrient counterparts (DiFeliceantonio et al., 2018). The current study extends these findings by isolating macronutrient-specific neural responses to orally delivered stimuli, indicating that these processes occur beyond just the valuation of visual cues associated with nutrients.

Although nutrient-specific reward valuation can be observed from the abovementioned results, an open question that remains is how these nutrient compositions are sensed. It also then stands to reason that the sensory parameters that contribute to the detection of these nutrients, specifically those pertaining to fat and sugar, would modulate the resultant subjective value of the oral food stimuli. As established previously, the presence of sugar is achieved through chemosensory mechanisms, whereas the results of this current project add to the already extensive body of evidence for fat detection occurring through textural parameters (de Araujo & Rolls, 2004; de Wijk & Prinz, 2007; Grabenhorst & Rolls, 2014; Kadohisa, Rolls, et al., 2005; E. T. Rolls, 2011; E. T. Rolls et al., 2018). Model comparison through AIC indicates that the GLMs using the taste parameter of sugar concentration and the texture parameters of viscosity and CSF marginally outperform those only using pure macronutrient content, indicating that these parameters are slightly better predictors. It is crucial to note that sugar content and sugar concentration are inherently the same in both models, as one is derived from the other, whereas fat content is reflected by an increase in viscosity and a decrease in CSF in all stimuli apart from the CMC stimulus. Therefore, with the exception of the CMC stimulus, both models essentially measure the same parameters, although the use of three parameters instead of two does not seem to impair the model's AIC due to the improved fit, implying that taste and textural parameters are indeed better predictors than pure nutrient value. Furthermore, trial-by-trial subjective psychophysical ratings

are substantially better predictors. This is likely due to the fact that models using these ratings as regressors take into account trial-by-trial variations in the internal state of the participants, such as satiety, attention and pre-existing amount of saliva in the oral cavity, thereby suggesting that subjective reward is determined by the nutrient-specific values that are sensed through taste and textural parameters and modulated by internal state.

The neural mechanisms behind reward valuation through the delivery of oral food stimuli can be considered from the results in Chapter IV. Specifically, the neural processing of BDM values, as proxies of subjective value, can be seen to be encoded strongly in the OFC upon receipt of the stimulus. Using a delayed onset of 2.5s, the decoding accuracy in the OFC is maintained with the addition of the vmPFC also encoding subjective value, suggesting that subjective value is first processed in the OFC before then being forwarded to the vmPFC for task-related processing. Rolls (2011) reviews the vast literature on the OFC implicating it in the processing of stimuli from all sensory modalities, focusing on taste and olfactory modalities in conjunction with textural properties, to compute an overall reward value for a stimulus (McCabe & Rolls, 2007; Padoa-Schioppa & Assad, 2008; Peters & Büchel, 2010; E. T. Rolls & McCabe, 2007; Rushworth, Behrens, Rudebeck, & Walton, 2007). Unlike responses in the taste and somatosensory cortices, OFC responses are modulated by homeostatic internal states such as sensory-specific satiety (B. J. Rolls, Rolls, Rowe, & Sweeney, 1981; B. J. Rolls, Rowe, et al., 1981; B. J. Rolls, Van Duijvenvoorde, & Rolls, 1984; E. T. Rolls et al., 1986; E. T. Rolls & Rolls, 1997). These studies implicate the OFC as the hub that integrates sensory and visceral signals from the appropriate primary processing structures in order to formulate a reward value that takes into account all of these properties. This property of the OFC can also be observed in the current thesis, where both viscosity and CSF signals can be found in the OFC, in addition to subjective value signals, as evidenced by the three-way conjunction analysis. However, the three-way conjunction analysis of viscosity, CSF and fat choice probability only highlighted a large cluster in the midposterior insula and the oral SSC, implying that the reward value being integrated in the OFC depends on the textural properties irrespective of the participants' conscious knowledge of the fat content of the stimulus. Taken together, these findings paint an overall picture of the integration of the reward value of fat texture as a component of oral food stimuli, where reward value is derived from different sensory modalities (visual, olfactory, taste and somesthetic) and integrated in the OFC.

5.4 – Roles of Specific Neural Structures

Specific brain structures previously known to be involved in chemosensory, somesthetic and reward processing of orally delivered food stimuli were also identified in this current project. In this section, we will explore these structures in more detail, discussing the roles they were known to play in oral food stimulus processing and reward integration based on the literature, in addition to how the results of the current work further advances the current body of knowledge. Although the original structures of interest can be found in Table 5.1, the results of the current project have strong implications for three specific neural structures in the context of their processing of oral food stimuli, namely the midposterior insula, the orbitofrontal cortex and the oral somatosensory cortex. Therefore, this section will discuss these three structures in most detail, showing how the results of the current work advance our understanding of their functions.

Table 5.1

Relevant Structures and Updated Functions

Region	Updated Function
Anterior Insula	Primary taste cortex, responding to chemical properties and viscosity of oral stimuli
Posterior Insula	Somesthetic processing of oral stimuli, encoding textural properties such as viscosity and sliding friction
Orbitofrontal Cortex	Processing of stimuli from various sensory modalities, including taste and oral texture, to integrate them into a reward value, specifically processes sliding friction information received from the oral SSC
Oral Somatosensory Cortex	Responsive to somesthetic information within and around the oral cavity, responsive to fat content of oral stimuli through textural properties such as viscosity and sliding friction
Lateral Hypothalamus	Appetite regulation, encouraging eating behaviour and processing visceral stimuli to drive eating and stop feeding when satiety is reached, informing the caloric content of food
Amygdala	Reward processing of stimuli from various sensory modalities, including taste and viscosity, planned pre-prandial behaviour
Ventromedial Prefrontal Cortex/Pregenual Cingulate	Reward valuation, receiving reward signals from areas in the reward system to formulate a decision, operates at a slight latency compared to the OFC due to being further downstream

5.4.1 – Insula

5.4.1.1 – Previously Known Functions

The insula has long been identified as a structure responsive to gustatory stimulation in the oral cavity. From even early work using single-neuron gustatory stimulation and ablation approaches in the insula, the presence of taste-sensitive neurons in the insular cortex and neighbouring frontal operculum was noted (Bagshaw & Pribram, 1953; Scott et al., 1986; Yaxley et al., 1990). Tractographical evidence points to differing afferent inputs into the agranular anterior insula and the dysgranular and granular posterior segments of the insula (Mufson & Mesulam, 1982), which also contributes to the distinct responses found in these structures. As discussed in the introduction chapter, the anterior agranular insula appears to be more responsive to chemosensory stimulation, whereas the dysgranular and granular portions towards the posterior are more

responsive to mechanical and somesthetic information in the oral cavity (Kadohisa, Rolls, et al., 2005; Kadohisa, Verhagen, et al., 2005; E. T. Rolls et al., 2018; Verhagen et al., 2004).

These specific roles of the distinct segments of the primate insular cortex have also been confirmed in humans through functional neuroimaging studies, where responsiveness to sweet taste, in addition to viscosity, elicits activations in the agranular anterior insula (de Araujo & Rolls, 2004). The sensitivity of this structure located here is such that even a reduction in the ionic concentration of the oral cavity through plain drinking water elicits activations in the primary taste cortex (De Araujo et al., 2003). While the presence of taste stimuli evoked activations in the agranular insula, activations in the granular insula are more pertinent to somesthetic information such as the viscosity of the oral food stimulus (de Araujo & Rolls, 2004). The posterior region of the insula has greater connectivity with more somatosensory areas (Cauda et al., 2011). Interestingly, while the anterior insula indicates the presence of taste stimuli, the processing of the pleasantness of oral stimuli also recruits the granular insula (Dalenberg et al., 2015), which implies that the posterior insula processes a quality of oral stimuli that is distinct from taste but is then integrated to contribute to their pleasantness. Since olfactory stimulation is processed more anteriorly, it is likely that the quality processed is the texture of the oral food stimuli. Recent stereoencephalography work on insulo-opercular processes involved in the tasting of food stimuli have shown that, when comparing a pleasant chocolate milkshake to a tasteless isotonic rinse solution, there is no localisation of insular population encoding between the anterior and posterior insula (Huang et al., 2021), although the large number of macronutrient differences between the stimuli used did not allow for the analysis of localised encoding of specific macronutrient components. Insulo-opercular signals related to anticipatory consumption of stimuli from cues were also elicited in the time frame leading up to consumption during an ad-libitum eating test, indicating that the insula is also involved in the expectation of food intake. Taken together, the two distinct roles of the anterior and posterior insular cortices seem to be the separate processing of taste and textural properties of oral food stimuli, both during consumption and anticipation of consumption.

5.4.1.2 – Contribution of Current Work

The current project fits very well within the context of previously known functions of the distinct insular cortices. Results from Chapter IV indicate that all parts of the insular

cortex are activated when receiving liquid food rewards as opposed to a tasteless rinse solution, in addition to implicating specifically the midposterior insula in the processing of the textural properties of oral food stimuli. Both the viscosity and the coefficient of sliding friction (CSF) of the stimuli were decoded from the BOLD activations in the insula, specifically posterior to and surrounding the limen insulae, which is similar to previous work on relating fat texture and viscosity to posterior insular activity (de Araujo & Rolls, 2004; Kadohisa, Verhagen, et al., 2005; E. T. Rolls et al., 2003; Verhagen et al., 2003). Due to the proximity between the posterior insula and the oral SSC, many clusters using univariate GLM and MVPA that passed the cluster-cutting threshold tended to spread across both structures. One such example is the positive contrast in the univariate GLM of CSF in the oral SSC that extends to the midposterior insula, although the MVPA results have more discrete clusters indicating more localised population encoding. As this project is the first functional neuroimaging work on humans into the CSF of orally delivered food rewards, these results tie in strongly with single-neuron recordings of correlates of CSF in macaques (E. T. Rolls et al., 2018), thereby implicating the midposterior insula as a structure that processes both the viscosity and CSF of oral food rewards.

In addition to the encoding of the CSF and viscosity of oral food stimuli, the posterior insula also appears to be involved in the judgement of fat content of these stimuli. That is, we are able to predict if participants would classify the oral stimulus as fat-containing from the posterior insular activation patterns upon oral delivery of the stimulus. These results have profound implications for the mechanisms of fat sensing, as they indicate that the pertinent sensory parameters used to determine the fat content of oral stimuli are processed in the posterior insula, from which participants draw on the required information about the fat content of foods. Given the role of this structure in textural processing (de Araujo & Rolls, 2004; E. T. Rolls, 2011), this strongly implies that fat content is sensed through mechanosensory and somesthetic perception, contrary to claims regarding the chemosensory nature of fat detection (B. V. Kulkarni & Mattes, 2014; Running et al., 2015; Tucker et al., 2014). Decoding accuracy in the posterior insula is also negatively correlated with the ability to correctly report CMC stimuli as fat-containing, implying that participants who are able to distinguish viscous non-fatty stimuli from fatty stimuli drew on the information encoded in this structure. As the existence of people who are able to orally distinguish fatty suspensions from simple viscous fluids has been used

to argue for the classification of fat taste as a primary taste (Running et al., 2015), these results suggest that participants who are sensitive to fat content are simply more finely tuned in the processing of fat texture, as they draw information from the posterior insula. It is likely that the ability to successfully differentiate fat and CMC solutions is a result of sensitivity to the discordance between fluctuations in viscosity and CSF, both of which are processed in the posterior insula. Therefore, the results of the current work extend both the understanding of the role of the posterior insula in the processing of oral food textures, especially CSF, and the understanding of how these textural properties are subsequently used in the determination of fat value of food in the oral cavity.

5.4.2 – Orbitofrontal Cortex

5.4.2.1 – Known Functions

The role of the orbitofrontal cortex in secondary sensory and reward processing has been documented since the early stages of primate neuronal ablation studies, where orbitofrontal ablations impair the ability to learn new assigned values (Butter et al., 1969). Due to convergence of afferent inputs into the OFC from various sensory modalities including taste, visual, somatosensory and olfactory modalities (Barbas, 1988; Barbas & Pandya, 1989; Baylis et al., 1995; Francis et al., 1999; Pandya et al., 1981; E. T. Rolls & Baylis, 1994), in addition to the discovery of task-related value signals in OFC neurons (Padoa-Schioppa & Assad, 2006), the OFC has been proposed as the structure where stimuli from various sensory modalities are integrated and computed into a coherent reward value (Kringelbach & Rolls, 2004; E. T. Rolls & Grabenhorst, 2008). Specifically in the context of oral food stimuli neuroimaging, the OFC has been shown to respond to the presence of oral fat and the resultant increase in viscosity (de Araujo & Rolls, 2004). The reward value of fatty stimuli has also been shown to be associated with activations in the OFC (Grabenhorst, Rolls, et al., 2010), while functional coupling between the OFC and the oral SSC increased during the ingestion of pleasant oral fat (Grabenhorst & Rolls, 2014). Much more recently, neurons encoding the CSF of oral food stimuli have also been identified in the OFC (E. T. Rolls et al., 2018), which were also identified as fat-responsive neurons that did not encode the viscosity of oral food stimuli (Verhagen et al., 2003), although this has yet to be shown to occur in humans.

In the context of nutrient-specific encoding, the lateral OFC also holds representations of the nutrient levels of visually presented food stimuli, whereas the

medial OFC is contains representations of the subjective value of the food (Suzuki et al., 2017). Furthermore, OFC reward responses to specific rewarding stimuli are attenuated after sensory-specific satiety (Charroud et al., 2021; De Araujo et al., 2003; S. Frank et al., 2010), which is possibly linked to afferent input from regulatory limbic areas such as the hypothalamus. Conversely, hunger increases OFC activation upon evaluation of calorie-dense foods (Siep et al., 2009), implying that the reward valuation processes in the OFC also take into account internal state to assign a final reward value of the food item. Therefore, it appears that the OFC can be thought of as a hub which integrates the various sensory and visceral information pertinent to assigning the reward valuation of the food item.

5.4.2.2 – Contribution of Current Work

Within the context of the literature of the function of the OFC in the multisensory integration of reward value of food items, the current project used the existing body of knowledge on reward integration, especially in the OFC, and expanded it further through the identification of CSF signals in the OFC. As seen from Chapter IV results, there is population encoding of both the viscosity and CSF of orally delivered stimuli in the OFC. Notably, the OFC was not highlighted in the univariate GLM of viscosity, whereas BOLD activations in the OFC were negatively correlated with the CSF of oral food stimuli. This suggests that the OFC has, as a whole, more voxels tuned to the reduction of CSF, which in turn would be used to indicate the presence of oral fat. This set of results is crucial on two points, namely that it echoes the recent primate work establishing that fat-sensitive OFC signals that are insensitive to viscosity actually encode the CSF of oral stimuli (E. T. Rolls et al., 2018) in the human population, and that the viscosity and CSF accuracy maps, though similar, ultimately show that they encode different parameters, as a perfect correlation between viscosity and CSF would result in similar univariate GLM results as well. Crucially, we also note the lack of OFC activation in the contrast between all stimuli and the tasteless rinse, implying that the OFC activations do not occur simply due to oral stimulation but in response to more specific parameters of oral stimuli.

Similar to the results of Grabenhorst and Rolls (2014), increased coupling between the OFC and the oral SSC was also found during consumption of oral stimuli with low CSF. As the previous study was conducted on the pleasantness of fat texture, and the current work has a greater focus on the textural parameters of the oral food stimuli, these

results suggest that the OFC draws on textural information from the oral SSC regarding the CSF of oral food stimuli, where it is then integrated into a unified reward value for the food. This is possibly a similar mechanism through which the OFC learns the fat content of food items, as it has also been recorded to reflect the macronutrient content of visually presented foods (Suzuki et al., 2017). Furthermore, the OFC is also the only structure which encodes both textural properties of the oral food stimuli in addition to the subjective value of the stimulus in the oral cavity upon delivery, as seen through the three-way conjunction of the viscosity, CSF and BDM maps. Interestingly, the encoding of subjective value persists even with a 2.5 second delayed onset, suggesting that the processing of reward value in the OFC may occur upon receipt of the oral stimulus and with a slight latency before forwarding the information to areas such as the vmPFC. This notion ties in well with the idea that the OFC is an integrator hub of various sensory and visceral modalities in order to form a subjective reward value (E. T. Rolls, 2011; E. T. Rolls & Grabenhorst, 2008). Given this context, the current project has expanded the span of somesthetic inputs into the OFC to include the encoding of the CSF of oral food stimuli, which had hitherto been unexplored in humans, in addition to confirming the latency of processing in the OFC, indicating its downstream position from the primary sensory and taste cortices.

5.4.3 – Oral Somatosensory Cortex

5.4.3.1 – Known Functions

As the name of the structure suggests, the oral SSC is the portion of the primary sensory cortex (SI) that is known to receive input from mechanical input from the lips and the oral cavity, including the tongue (Kaas, 1983; Merzenich et al., 1978). This portion of the SI has been shown to span a large area of the cortex, with rich and detailed representations of mechanical and thermal inputs through the projection of C fibres originating from the oral cavity and passing through the trigeminal nuclei and thalamus (Cerkevich et al., 2013, 2014). Notably, these inputs are separate to taste-sensitive fibres that are projected through the parvocellular ventral posterior nucleus (VPMpc), indicating that these inputs correspond solely to mechanical stimulation rather than taste stimulation. The rich mapping of the oral SSC has been confirmed through electrophysiology and histology, with a rostrocaudal convergence from initially large receptive fields (Toda & Taoka, 2001, 2004), such that the oral SSC itself covers a large

portion of the cortex. This large receptive field is indeed observed in the literature, where various neuroimaging studies show clusters centred in different parts of this large area (de Araujo & Rolls, 2004; Eldeghaidy et al., 2011; Miyamoto et al., 2006; Pardo et al., 1997; Veldhuizen et al., 2011; G.-J. Wang et al., 2002) such that Grabenhorst and Rolls (2014) averaged these largely disparate coordinates to analyse the cortical representation of the textural properties of fat. Taken together, these studies point to the oral SSC as a vast cortical structure that is highly responsive to somesthetic information of the oral cavity, thereby making it the primary structure through which textural information on oral food stimuli is processed.

5.4.3.2 – Contribution of Current Work

The current project reinforces the well-established idea in the literature of the oral SSC being one of the primary cortical structures involved in the processing of the textural properties of oral food stimuli. The fMRI results in Chapter IV implicate the oral SSC in the processing of orally delivered fatty stimuli. Moreover, the oral SSC also encoded the viscosity and CSF of orally delivered stimuli, as seen from the MVPA decoding results. Interestingly, due to the large area of cortex covered by the oral SSC, the univariate GLM results highlight parts of the bilateral oral SSC in the positive CSF contrast, whereas a negative contrast implicated a more superficial and dorsal region of the SI which is still arguably within the limits of the oral SSC. From these contrasts, we may deduce a ventrodorsal gradient in the oral SSC of neurons tuned positively to negatively to the CSF in the oral cavity. These large clusters also appear to hold a population-level encoding of both viscosity and CSF, further supporting the proposed role of the oral SSC in the processing of the textural properties of food.

One crucial finding from this project on the oral SSC is, beyond the simple presence of fat in the oral cavity, participants draw on the information encoded in the oral SSC in order to determine the fat content of the food stimulus in the oral cavity, regardless of the accuracy of this choice. This is indicated by the ability of the SVM decoder to predict the participants' fat rating of the stimulus from the BOLD activation patterns in the oral SSC upon delivery of the stimulus. Furthermore, an SVM regressor is able to predict the probability that each stimulus from the set of stimuli is marked as fat-containing from the activation patterns in the oral SSC. These results are pivotal in the sense that they suggest that humans use information from the oral SSC to base their decisions on the presence of

fat in oral food stimuli and, extrapolating from the known role of this structure in the specific processing of mechanosensory and somesthetic information of the oral cavity, further support the idea that fat detection of oral food stimuli occurs through textural means. Indeed, this idea is reflected in the three-way conjunction of the viscosity, CSF and fat choice probability maps, where the oral SSC is the only structure that contains the representations of all three measures at the time of stimulus delivery and thereby suggesting that the oral SSC is the structure that contributes to fat valuation in oral stimuli irrespective the reward value assigned to the stimulus.

5.4.4 – Lateral Hypothalamus

5.4.4.1 – Known Functions

The hypothalamus is known to regulate feeding behaviour by signalling of satiety levels through leptin-induced signalling (Elmquist et al., 1998, 1997). In its function as a regulator for feeding behaviour, the hypothalamus signals food-related cravings that can be attenuated by oral ingestion of glucose (Smeets et al., 2007). In addition, BOLD activations in the hypothalamus are also elicited during the consumption of oral fat, irrespective of the pleasantness of the food stimulus (Grabenhorst, Rolls, et al., 2010). Therefore, the hypothalamus can in general be taken to respond to basic metabolic cues, regardless of the specific nutrient component of the food ingested.

5.4.4.2 – Contribution of Current Work

The findings of this project have largely supported the idea of the hypothalamus as being involved in the regulation of eating behaviour. The hypothalamus most prominently featured in a contrast between all stimuli and the tasteless rinse, indicating that the consumption of an oral food stimulus is responsible for BOLD activations in the hypothalamus. At the point of stimulus delivery, hypothalamic activations are functionally coupled with oral SSC activations, indicating related processes. Indeed, the hypothalamus can also be seen in to encode the textural parameters of fat, namely viscosity and CSF, which may have contributed to hypothalamic activity to oral fat levels (Grabenhorst, Rolls, et al., 2010). However, as the hypothalamus is a relatively small subcortical region with specialised nuclei (Herrick, 1908, 1910), signal detection from specific nuclei using fMRI is often difficult (Macey, Ogren, Kumar, & Harper, 2016), such that it would be prudent to refrain from drawing strong conclusions based on the absence of results in certain contrasts.

5.4.5 – Amygdala

5.4.5.1 – Known Functions

The amygdala has also been implicated in the consumption of oral stimuli, as amygdalar signals have been reported to correspond to the viscosity of oral stimuli in single-neuron macaque studies (Kadohisa, Rolls, et al., 2005; Kadohisa, Verhagen, et al., 2005). Human neuroimaging studies have also demonstrated BOLD activations in the amygdala in response to oral fat content (Eldeghaidy et al., 2011; Grabenhorst, Rolls, et al., 2010). Zangemeister, Grabenhorst and Schultz (2016) also showed that the amygdala encodes future saving plans for food rewards that are delivered imminently in the scanner, closely tying the role of the amygdala with an ecologically valid prospective reward outcome of food-related tasks. Therefore, the role of the amygdala in food reward valuation is complex, as it both encodes the individual component properties of the food stimuli (such as fat and viscosity) in addition to the future planning of specific food choices (Zangemeister et al., 2016).

5.4.5.2 – Contribution of Current Work

The results from Chapter IV implicate the amygdala in the encoding of textural and taste properties of oral food stimuli. Specifically, population encoding of the viscosity of oral food stimuli can be found in the amygdala at the time of stimulus delivery, although no results were found in the amygdala for the CSF map. Interestingly, the amygdala is more featured when decoding based on subjective ratings, with amygdala activation patterns allowing the decoding of subjective ratings of sweetness and oiliness, whereas a univariate GLM showed positive encoding of subjective thickness ratings in the amygdala. Although the only physical parameter which was shown to be encoded in the amygdala was viscosity, as with the hypothalamus the nature of the amygdala's heterogeneous neuronal population with small nuclei corresponding to specialised functions encourages caution in the interpretation of negative results. Furthermore, as noted in Chapter III, subjective psychophysical ratings tend to provide a better description of each participant's internal state, such that the use of subjective ratings may have been more sensitive, thus leading to signals being noted in the amygdala for these ratings.

5.4.6 – Ventromedial Prefrontal Cortex/Pregenual Cingulate

5.4.6.1 – Known Functions

The ventromedial prefrontal cortex (vmPFC)/pregenual cingulate has often been linked to reward value, especially in the case of neuroimaging of rewards (Chib et al., 2009; Peters & Büchel, 2010). Pregenual cingulate signals have been linked to pleasantness ratings of both oral food reward and touch stimuli at a pleasant temperature (Grabenhorst, D'Souza, Parris, Rolls, & Passingham, 2010; Grabenhorst, Rolls, et al., 2010). Unlike the OFC, value encoding in the vmPFC appears to be linked to the overall value of the reward given without specifically encoding the identity of these rewards (Howard et al., 2015). Formal economic valuations using the BDM auction task (Becker et al., 1964) have frequently shown encoding of value in the vmPFC, which is distinct from the OFC as this encoding does not in itself contain information on the components of the reward value, such as the nutrient content of visually presented foods (DiFeliceantonio et al., 2018). Nutrient-specific information also appears to be absent in value encoding in the medial OFC, which is found between the lateral OFC and the vmPFC, suggesting that the lateral OFC forwards information on reward component to the medial OFC and the vmPFC.

5.4.6.2 – Contribution of Current Work

The results of Chapter IV implicate the vmPFC in subjective ratings, most notably the subjective value as measured using the BDM auction task. Crucially, vmPFC encoding of BDM values was only possible after using a delayed onset of 2.5s, whereas the OFC encoding of the BDM values persisted from the onset, with a more medial peak. This latency is likely to indicate that the vmPFC receives integrated input of the identity-specific reward value from the OFC, after which it is expressed in an identity-general manner in the vmPFC (Howard et al., 2015). This lack of specificity to reward identity in the value signals of the vmPFC can be deduced from the lack of vmPFC encoding of taste or textural parameters. These results are in line with the literature where the lateral OFC is seen to encode individual reward components and the medial OFC and vmPFC/pregenual cingulate reflect the reward value irrespective of components (DiFeliceantonio et al., 2018; Suzuki et al., 2017; Tang et al., 2014). Most notably, this project is, to our knowledge, the first to show vmPFC encoding of formally tested, incentive-compatible reward value of orally delivered food stimuli at the point of

ingestion, as previous research into food reward have focused on visual stimuli (DiFeliceantonio et al., 2018), used pleasantness ratings (Grabenhorst, D'Souza, et al., 2010; Grabenhorst, Rolls, et al., 2010) or noted vmPFC signals during BDM tasks performed before the consumption of orally delivered liquid food rewards (Zangemeister et al., 2016).

5.5 – Implications of Fat Detection Mechanisms

The findings of this thesis have shed light on the neural mechanisms of fat sensing, specifically the textural mechanisms involved therein, and how fat detection contributes to the formation of reward value for a food item. From the behavioural results, one notes that macronutrient content, in the form of fat and sugar, is indeed a predictor of reward value and that the subjective value component of fat is derived from textural parameters that are processed in both the oral SSC and the OFC. In addition to furthering our knowledge of these fundamental biological processes, this project also identified potential implications of the work in a wider context, as will be explained in this section.

5.5.1 – Clinical Implications

The neuroimaging results of this thesis highlights the orbitofrontal cortex as the main hub of reward value processing, where information from various modalities is integrated into a cohesive reward value for the food item. The sensitivity of OFC neurons to textural parameters in particular highlights the importance of food texture in the formation of reward value, which is a parameter often left unexplored in most functional neuroimaging studies. Indeed, a number of studies on reward integration of food items in the orbitofrontal cortex rely on the visual presentation of such stimuli (DiFeliceantonio et al., 2018; S. Frank et al., 2010; Stoeckel et al., 2008; Suzuki et al., 2017), which by necessity neglects the processing of real-time textural information of the food items. It is, however, this sensitivity to the textural parameters of oral food stimuli that is pivotal in this mechanism, as dysfunction in this reward integration in the OFC may lead to maladaptive outcomes. For example, hypersensitisation of the OFC to viscosity and CSF signals received from the oral SSC might lead to consistent overvaluation of high-fat foods, which in the long term is likely to result in an energy intake imbalance. Obese individuals display greater oral SSC activations upon receipt of a pleasant chocolate milkshake than lean controls (van der Klaauw et al., 2014), which is likely to increase OFC value signals related to the reward value of the received food stimulus. This would

subsequently result in the consistent overvaluation of high-fat stimuli that are, assuming other macronutrients are kept constant, high in caloric density, contributing in part to increasing world obesity levels (Brunstrom et al., 2018; Skorupa et al., 2008).

5.5.2 – Industrial Implications

In addition to the clinical implications, the current work also has implications for the food industry, especially within the context of fat intake and fat replacement. The popularity of high-protein low-fat foods continues to increase as the general population aims to limit the consumption of calories while still maintaining their previous levels of satiety (Sandrou & Arvanitoyannis, 2000). It is therefore crucial that low-fat substitutes mimic both the neural and behavioural effects induced by their high-fat counterparts in order to effect long-lasting change in dietary patterns. One interesting finding regarding fat intake uncovered by this project is that the extent to which fat determines subjective value in the behavioural pre-testing task using orally delivered liquid rewards also predicts the percentage of fat eaten in the ad-libitum eating test. In addition to demonstrating the ecological validity of the pre-testing task, this correlation also shows that individual fat preference persists through different dishes (liquid milkshakes to more solid curries) and different types of meals, as milkshakes are generally consumed as a dessert or snack item whereas curries tend to form the main portion of a meal. Crucially, the eating task also revealed that individual subjective value of the CMC fat replacer stimulus is negatively correlated with fat intake, implying that participants with greater acceptance of CMC eat less fat in a standard meal. As CMC has been used in the food industry to increase viscosity of low-fat foods since as early as the 1940s (Hollabaugh, Burt, & Walsh, 1945), it is one of the most commonly used fat replacement hydrogel polymers in the food industry. However, the results of the ad-libitum eating test clearly indicate that participants who consume more fat in a standard meal are not receptive to CMC replacement in liquid stimuli, which suggests that currently prevalent fat-replacement strategies are aimed at the wrong demographic. While conscious positive attitudes to fat-replacement have been negatively correlated with fat intake in general (Stafleu, De Graaf, Van Staveren, & De Jong, 1994), the results of the current study suggest that this may be tied to individual sensitivity to fat textural properties that are not currently faithfully emulated in low-fat foods.

Due to increasing knowledge of ideas such as protein-leveraging (Simpson & Raubenheimer, 2005), in addition to the more widespread understanding of the higher satiety induced by high-protein diets (Crovetti et al., 1998; Eisenstein et al., 2002; Veldhorst et al., 2008), there has been a surge in demand of high-protein low-fat foods, such as Greek yoghurt. The high protein content of these foods tend to also result in high viscosities, such that the average viscosity of most Greek yoghurts are around 21000 cP (Behnia, Karazhiyan, Niazmand, & Mohammadi Nafchi, 2013). Despite these high viscosities, the lack of fat content of these foods is apparent, likely due to high CSF values (Laguna et al., 2017). In these products, the CSF increase may be attenuated through the addition of another liquid, such as soya milk, to reduce the structural integrity of such foods and reduce CSF without additional fat content. Alternatively, another water-based additive may be added that may reduce CSF while minimising viscosity. Pullulan may be a candidate for such an addition, as its addition to an aqueous solution is known to reduce the resultant CSF, especially in the presence of sodium chloride or sodium fluoride (Xu et al., 2017). Such additions may mitigate the viscosity-CSF discrepancy that is likely to be the cause behind the rejection of low-fat foods by some fat-sensitive individuals.

5.6 – Limitations

Contributions to current knowledge base of the textural properties of oral food stimuli and fat content notwithstanding, the current project is by no means without its limitations. One such limitation was the limited number of participants recruited. Although a total of 31 participants took part in the behavioural pre-testing, COVID-related lockdowns and restrictions resulted in only 23 participants being scanned, where one dropped out during the experiment such that only 22 viable fMRI datasets were acquired. Moreover, only 16 participants completed the ad-libitum eating tasks, as 3 were unable to be tested due to the COVID-related closure of the Translational Research Facility, 1 was lost to follow-up and two received the wrong stimulus set. This low number of participants limits the conclusions that can be drawn from the study, especially those pertaining to the real-life eating behaviour of participants. The timing of this study, with the behavioural pre-testing, scanning and real-life eating test at different centres all within 15 days of each other, ensured the validity of the test as participants were unlikely to have changed their eating behaviour or preferences in such a short time, although these complicated logistics also resulted in difficulty given the different policies of the

three centres on testing during the COVID-19 pandemic, resulting in unforeseen limitations on the project that were difficult to resolve.

Another limitation of the study was the low number of stimuli used. This limit of seven experimental stimuli, and a rinse solution, arose due to necessity, as the required number of repetitions and the event-related design with long tasting periods both meant that participants had to undergo MRI scanning for an extended period of time. Further introduction of novel stimuli would have resulted in longer scanning times, which may have been intolerable for the safety and comfort of the participants. Nevertheless, extensive pre-testing and optimisation ensured that the stimulus set used was able to elicit the desired responses from participants, such that the fMRI results were able to demonstrate the role of textural parameter processing in the detection of fat content of orally delivered stimuli. However, the results would have been improved with either a viscosity or CSF series to break the intercorrelation of the two parameters.

5.7 – Future Directions

The current project has opened several possible avenues for further research. One such key research topic would involve the use of both a viscosity series and a CSF series, maintaining the converse parameter as constant as physically viable, in order to truly tease apart their separate neural encoding. This may be coupled with a fat-detection task using high fat stimuli as well, where concordant viscosity and CSF values are presented alongside discordant values – that is, stimuli high in viscosity and low in CSF and vice versa – to explore individual variations in fat detection capabilities. Alternatively, the individual ideal viscosity and CSF values of foods may be computed and recreated in order to create a uniquely hyperpalatable food stimulus for each participant. Comparing these results with another real-life eating test would indicate if participants who value one textural parameter over another has specific real-life eating characteristics, in addition to exploring the effect of improving the textural concordance of low-fat food items on fat detection in individuals who are sensitive to fat content.

The current project also focused on the nutrient-specific component of eating behaviour. However, eating behaviour has strong cultural and social components that drive it (Barthes, 2012; Kittler, Sucher, & Nelms, 2016; K. D. Kulkarni, 2004), which have largely been unexplored in this project. Given the role of structures such as the amygdala in predicting others' food choices and reward value in macaques (Grabenhorst & Schultz,

2021), the existence of a similar process in humans would explain the social effects of food choices through the learning of others' assigned reward values to different foods. It would be interesting to explore the extent to which others' choices influence one's food preferences in such a setting, as well as how the amygdala may play a role in this process.

Appendix A – TRF Testing Information and Questionnaires

Breakfast Options

1	2 slices of toast with jam/marmalade and small glass of orange juice		
	Thin White Sliced Bread	75	g
	Jam/Marmalade	12	g
	Breakfast/fresh orange juice	100	ml
2	Bowl of Cereal with semi skimmed milk		
	Cornflakes	50	g
	Semi Skimmed Milk	140	ml
3	Bowl of Granola with small pot of natural yoghurt and handful of fruit		
	Tesco Greek Yoghurt	100	g
	Tesco Granola	26	g
	Blueberries	30	g

Code: **Date:**

Pre-test Questionnaire

Did you eat breakfast today?	Y / N
Did you follow the recommended breakfast guidelines?	Y / N
Have you eaten anything since?	Y / N
If yes, please list what you ate:	
Do you normally eat breakfast?	Y / N
Do you normally eat lunch?	Y / N
How many hours ago did you have something to drink (except water)?	
Approximately how much water have you had today? (1 cup = 150 ml)	
How hungry are you now? (1= not hungry; 10 = very hungry)	
How thirsty are you now? (1 = not thirsty; 10 = very thirsty)	

Code: **Date:**

Solid Food Taste Ratings

	A	B	C
Sweetness (0 = not sweet; 10 = very sweet)			
Thickness (0 = not thick; 10 = very thick)			
Pleasantness (0 = not pleasant; 10 = very pleasant)			
Oiliness (0 = not oily; 10 = very oily)			
Saltiness (0 = not salty; 10 = very salty)			
Savouriness (0 = not savoury; 10 = very savoury)			

Code: **Date:**

Post-test Questionnaire

How hungry are you right now?

1	2	3	4	5	6	7	8	9	10
Not hungry									Very hungry

How thirsty are you right now?

1	2	3	4	5	6	7	8	9	10
Not thirsty									Very thirsty

How big is your appetite?

1	2	3	4	5	6	7	8	9	10
small									big

Are you currently trying to restrict your food intake? Y / N

How restrictive are you when it comes to food?

1	2	3	4	5	6	7	8	9	10
relaxed									restrictive

On average, how much do you enjoy sweet foods?

1	2	3	4	5	6	7	8	9	10
Not at all									Very much

On average, how much do you enjoy fatty foods?

1	2	3	4	5	6	7	8	9	10
Not at all									Very much

What type of milk do you usually buy? (Please tick as appropriate)

Whole 4% milk (blue)	2% milk (green)	Skimmed milk (red)	Non-dairy milk	I don't drink milk	Other (please elaborate)

How often do you consume foods containing zero-calorie/reduced-calorie sweeteners? (e.g. aspartame, xylitol, stevia, maltitol in reduced-calorie sodas)

1	2	3	4	5	6
Never/Almost never	Less than once a month	Every month	Every week	Every day	More than once a day

How much are you actively trying to limit your sugar consumption?

1	2	3	4	5	6	7	8	9	10
Not at all									Very much

How much are you actively trying to limit your fat intake?

1	2	3	4	5	6	7	8	9	10
Not at all									Very much

Have you ever tried to limit your sugar intake? Y / N

Have you ever tried to limit your fat intake? Y / N

How representative was the lunch you just had of the amount you would usually eat?

1	2	3	4	5	6	7	8	9	10
Not at all									Very much

Appendix B – Participant Information Sheet

Information sheet

Neuroimaging of reward processing

We are inviting you to participate in a scientific fMRI study of brain function. This will not be of direct benefit to you and we do not wish you to feel under any pressure whatsoever to take part. Please feel free to refuse if you have any worries that remain after we have answered any questions that you may have.

What are MRI and fMRI?

MRI is an acronym for ' Magnetic Resonance Imaging', fMRI for 'functional Magnetic Resonance Imaging'. These are techniques that enable us to examine the structures of the brain in conjunction with their function by using an MRI scanner and analyzing the information obtained. MRI and fMRI are unique tools for research, and allow brain functions and behaviour to be investigated in terms of activation and blood flow of specific areas of the brain. The basis of MRI is the use of magnetic fields to produce a map of the water concentrations in the body. Within the bore of the scanner there is a very large uniform magnetic field. The person being scanned is moved on a bed into this magnetic field with their head inside a coil, which has the appearance of a large helmet. When the person's head is in the centre of the magnetic field, magnetic field gradients are switched on and off very rapidly to produce a signal for MR image formation. These produce a loud knocking noise throughout the scan.

Why is this study being done?

The study is being done to try and find out more about the effects of receiving reward on brain functions. In this experiment you will perform a rating task and consume small amounts of liquid food stimuli. The Cambridge Research Ethics Committee has given this study a favourable opinion.

What will I need to do?

You will be asked to visit the Department of Physiology, Development and Neuroscience on one occasion (before scanning ~ 60 min), the Wolfson Brain Imaging Centre (WBIC) at Addenbrooke's Hospital on another occasion (scanning ~ 90 min), and the Translational Research Facility (TRF) at Addenbrooke's Hospital on another occasion (after scanning, ~60 min). Before scanning, there will be a short questionnaire about your medical history. Please note that all information will be treated in the strictest confidence. We will then give you instructions about the task you are going to perform and once everything is clarified you will be lying as comfortable as possible in a tube designed to measure your brain activity while you perform the task. You will not be asked to take any drugs.

What are the possible risks of taking part?

Because of the use of strong rapidly changing magnetic fields, people who have implants such as cardiac pacemakers, aneurysm clips in their brain, cochlear implants, permanent eye-lining or anyone who has been exposed to metallic flakes or splinters travelling at high speed,

Fabian Grabenhorst (email: fg292@cam.ac.uk)
Department of Physiology, Development and Neuroscience, Downing St., Cambridge CB2 3DY

cannot be scanned. On all other people, brain-imaging techniques have been used safely for many years, both for scientific and clinical purposes. No side effects have been reported. The tube you will be lying in is rather narrow and the noise produced by the machine is rather loud. If you find this too unpleasant, the procedures would be stopped immediately.

What else do I need to know?

It is important to note that this study will remain completely voluntary at all times. If you do not wish to participate, or wish to stop participating at any stage you will be able to do so without having to explain why. Your decision will not affect any future treatment you might require from the health service. You will not be identified by name in any report concerning the study. It will not be necessary to contact your General Practitioner. Like faces, brains come in all shapes and sizes, so that there are many normal variations of what the scan shows. There is a chance of less than 1:100 that your MR scan may show a significant abnormality of which you are unaware. In such circumstances, you will be appropriately counselled. You will be referred to the appropriate specialist in consultation with your General Practitioner if that is what you would like. Such early detection has the benefit of starting treatment early but, in a small number of cases, may have implications for future employment and insurance.

Confidentiality – who will have access to the data?

The data will be stored on a secure network and only members of the WBIC and members of the research group will have access to the data. It is possible that the data may be used by researchers working with the WBIC for other similar ethically approved research protocols, where the same standards of confidentiality will apply. It may also be disclosed to researchers working outside the EEC, when that person is working in close collaboration with researchers scanning within the WBIC. In that case that person has signed a Code of Conduct guaranteeing that the data will be kept confidential and securely. The University is deemed to be the Data Controller and all enquiries concerning access to the data should be addressed to him. The Administrator of the Centre will be able to tell you the name and address of this officer.

What will happen to the study results?

They will be kept securely for a minimum of 10 years and possibly indefinitely in the WBIC data archive in accordance with good research practice.

Are there compensation arrangements if something goes wrong?

In the unlikely event of anything untoward happening, insurance has been taken out with Newline Insurance Company Ltd and Royal & Sun Alliance Insurance to cover this study.

If you would like more information.

Thank you for taking part in the study. We encourage you to think about the points made on this information sheet. We would be very happy to answer any questions you might have.

Fabian Grabenhorst (email: fg292@cam.ac.uk)
Department of Physiology, Development and Neuroscience, Downing St., Cambridge CB2 3DY

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