Neuropsychopharmacology of Appetite

in Healthy Volunteers

By

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A thesis submitted to

The University of Birmingham

For the degree of

DOCTOR OF PHILOSOPHY

School of Psychology

The University of Birmingham

September 2014

ABSTRACT

The aim of this thesis was to develop an Experimental Medicine Model for the assessment of the potential efficacy and psychiatric safety of novel anti-obesity drugs. Chapter 1 provides a general introduction to the research area. Chapter 2 presents evidence that the satiety enhancing effects of an appetite suppressant drug (meta-chlorophenylpiperazine – mCPP) can be detected after acute administration to healthy women participants using a Universal Eating Monitor (UEM) that provides measures of eating microstructure such as eating rate. Assessment of emotional responses using the P1vital® Oxford Emotional Test Battery **(**ETB) suggested an absence of psychiatric side-effects induced by mCPP at the doses tested. Chapter 3 reports that eating responses measured by the UEM are similar whether or not the participants are aware that their eating behaviour is being assessed, indicating that the practical limitations associated with assessing meal microstructure covertly can be avoided. Chapter 4 presents two studies which provide evidence that 1) repeated use of the ETB is feasible, thereby enabling it to be used in cross-over experimental designs and 2) performance on the ETB is not affected by satiety. In Chapter 5, functional magnetic resonance imaging (fMRI) was used to identify a profile of natural satiety against which the effect of drugs that enhance satiety can be compared. Chapter 6 reports the results of a study that combined the use of the UEM with fMRI to examine the effects of mCPP on behavioural and neural markers, in a cross-over design. mCPP significantly reduced blood oxygen level dependent (BOLD) activations to the sight of high calorie food images in key appetitive and reward areas and reduced consumption of a palatable cookie snack but did not decrease the intake of a pasta meal. Analysis of individual differences in responses to mCPP revealed that BOLD activity in reward-related brain areas predicted an anorectic response to drug administration. Chapter 7 provides a reflection on the experimental work and it is concluded that the proposed Experimental Medicine Model may be valuable for the development of efficacious and safe appetite suppressant drugs by providing additional data to inform go/no go decisions on drugs in early phase clinical trials.

STEVE COOPER PHD STUDENTSHIP

This thesis was completed in honour of the late Professor Steven J. Cooper. Professor Cooper was an internationally renowned researcher recognised for his work on the psychopharmacology of ingestive behaviour.

His research legacy continues with the many students he supervised, with those who are actively engaged in research in ingestive behaviour, and now, with the body of research contained within this thesis.

While I was not fortunate enough to have met Steven in person, I am deeply privileged to have completed this work in honour of his memory.

ACKNOWLEDGMENTS

I would like to thank Dr Suzanne Higgs and Dr Colin Dourish. It has been a privilege and pleasure to work with you both, and I am deeply grateful for your support and encouragement. You are my role models, and I hope to make you proud.

To my mother, sister and brother-in-law, I am deeply grateful for your support, and the persistent reminders that I am of Welsh descent.

Thanks also to Dr Michelle Lee who nurtured my love of Psychology in my (distant) youth.

And to Sean Russell who has become a very dear friend during my PhD, and reminded me of the importance of cake.

To the rest of my family and all of my friends and colleagues, thank you for your love and support.

I cannot thank my husband Kyle Reiss Thomas-Hadley, as words are far too inadequate to convey the appropriate level of gratitude. I love you deeply, more than anything else in the world (even chocolate), and this thesis is as much your accomplishment as it is mine.

I'll finish by dedicating this thesis to the memory of my father, Michael Thomas, who left me with the dark sense of humour I constantly inflict on everyone.

Financial support was provided by the University of Birmingham and P1vital.

DISSEMINATION

The contents of this thesis have been presented at the following conferences: The British Feeding and Drinking Group (2011, 2012, 2013 and 2014), Treatment of Obesity Conference (2012), The Society for the Study of Ingestive Behaviour (2012), The British Association of Psychopharmacology (2012, 2013 and 2014), the European Behavioural Pharmacology Society (2013) and the American College of Neuropsychopharmacology (2012, 2013 and 2014).

Study 1 from Chapter 2 has been published:

Thomas, J. M., Dourish, C. T., Tomlinson, J. W., Hassan-Smith, Z., & Higgs, S. (2014). Effects of the 5-HT_{2C} receptor agonist meta-chlorophenylpiperazine on appetite, food intake and emotional processing in healthy volunteers. *Psychopharmacology (Berl)*, *231*, 2449-59.

Study 5 from Chapter 6 has been published:

Thomas, J. M., Higgs, S., Dourish, C. T., Hansen, P., Harmer, C. J., & McCabe, C. (2015). Satiation attenuates BOLD activity in brain regions involved in reward and increases activity in an inhibitory control area: an fMRI study in healthy volunteers. *American Journal of Clinical Nutrition*. DOI: 10.3945/ajcn.114.097543

TABLES AND FIGURES

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GLOSSARY

5-HT	5-hydroxytryptamine - Serotonin
5-HTTLPR	Serotonin-transporter-linked polymorphic region
6-OH-DA	6-hydroxydopamine
ACC	Anterior cingulate cortex
ACQ	Alcohol and Caffeine questionnaire
AgRP	Agouti-related peptide
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
ARC	Arcuate nucleus
BDI	Beck Depression Inventory
BFS	Befindlichskeit scale of mood and energy
BIS 11	Barratt Impulsivity Scale
BIS/BAS	Behavioural Inhibition/Approach Scales
BMI	Body mass index
BOLD	Blood oxygen level dependent
BUIC	Birmingham University Imaging Centre
CBT	Cognitive behavioural therapy
CCK	Cholecystokinin
CNS	Central nervous system
COPE	Contrast of parameter estimate
CQ	Chocolate Questionnaire
dlPFC	Dorsolateral prefrontal cortex
DMH	Dorsomedial hypothalamus
dmPFC	Dorsomedial prefrontal Cortex
EAT	Eating Attitudes Test questionnaire
ECAT	Emotional Categorisation Task

ECG	Electrocardiogram
EEG	Electroencephalography
EMA	European Medicines Agency
EMEM	Emotional Recognition Memory Task
EPQ	Eysenck Personality Questionnaire
EREC	Emotional Recall Task
ETB	P1vital® Oxford Emotional Test Battery
FCPS	Fawcett-Clarke Pleasure Scale
FDA	Food and Drug Administration
FDOT	Faces Dot Probe Task
FERT	Facial Expression Recognition Task
FLAME 1+2	FMRIB's Local Analysis of Mixed Effects 1+2
fMRI	Functional magnetic resonance imaging
FMRIB	Oxford Centre for Functional Magnetic Resonance Imaging of the Brain
FSF	fMRI Screening Form
FSL	FMRIB software library
FTO	Fat mass and obesity-associated
FWE	Family wise error
GABA	Gamma-aminobutyric acid
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
GSTFT	Guy's and St Thomas' NHS Foundation Trust Pharmacy Manufacturing Unit
HH	High Fat / High Sugar
HL	High Fat / Low Sugar
LH	Low Fat / High Sugar
LH	Lateral hypothalamus
LL	Low Fat / Low Sugar

MCFLIRT	FMRIB's Linear Image Registration Tool
mCPP	meta-chlorophenylpiperazine
MEG	Magnetoencephalography
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components
MFB	Medial forebrain bundle
MNI	Montreal Neurological Institute
NAcc	Nucleus accumbens
NART	National Adult Reading Test
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NMDA	*N*-Methyl-D-aspartate
NPY	Neuropeptide Y
NTS	Nucleus of the solitary tract
OFC	Orbitofrontal cortex
PANAS	Positive and Negative Affective Schedule
PCA	Principal components analysis
PCC	Posterior cingulate cortex
PET	Positron emission tomography
PFC	Prefrontal cortex
PFS	Power of food Scale
POMC	Pro-opiomelanocortin
PVN	Paraventricular nucleus
PYY	Peptide YY_{3-36}
SCID	Structured Clinical Interview for DSM-IV Axis I Disorders
SE	Standard error
SHAPS	Snaith-Hamilton Pleasure Scale

SIPM	Sussex Ingestion pattern Monitor
SQ	Satiation quotient
SRQ	Sandwich Rating Questionnaire
SSRT	Stop Signal Reaction Time Task
SSS	Sensory specific satiety
STAI	State Trait Anxiety Inventory
TFEQ	Three Factor Eating Questionnaire
UEM	Universal Eating Monitor
V1, 2 & 4	Visual area 1, 2 & 4
VAS	Visual analogue scale
vlPFC	Ventrolateral prefrontal cortex
VMH	Ventromedial hypothalamus
vmPFC	Ventromedial prefrontal cortex
VTA	Ventral tegmental area
WTCRF	Welcome Trust Clinical Research Facility

CHAPTER 1: GENERAL INTRODUCTION

1.1. Obesity

1.1.1. Introduction

Obesity is a worldwide health priority because it is associated with an increased risk of coronary heart disease, hypertension, type II diabetes, and cancer (Finucane et al. 2011). It is also associated with a wide spectrum of co-morbidities, both physiological and psychological (e.g. depression), and is linked to early mortality (Brown et al. 2009). Therefore, obesity and its consequences present a massive public health challenge. Obesity is associated with excessive fat accumulation and is defined currently as body mass index (BMI - kg/m^2) > 30. However, there is evidence that waist circumference might be a more reliable indicator of health risk than BMI (Janssen et al. 2004) and that waist-to-height ratio might be an even more effective measure (Ashwell et al. 2013).

Data from the World Health Organization (WHO) showed that in 2008, more than 10% of the world's adult population were obese (WHO, 2012). In the UK in 2008, almost 25% of adults in England were obese, with close to 30% of boys and girls aged 2-15 classed as overweight-obese (Health and Social Care Information Centre, 2009). More recent data suggest that the increase in obesity rate may be slowing in children; possibly due to successful prevention campaigns (Health and Social Care Information Centre, 2014). Nevertheless, overall rates of obesity are projected to rise over the next decade, and by 2020 it is predicted that 70% of British people will be overweight or obese (Sassi, 2010). This is against a backdrop of no national success stories in tackling obesity in the past 33 years (Ng et al. 2014).

There is a significant financial burden associated with obesity because the treatment of obesity and its co-morbidities incurs both direct costs in terms of healthcare and indirect costs associated with premature mortality and loss of earnings due to ill health. In 1998, the direct and indirect costs of obesity in England were estimated to be £497 million and £2.6 billion respectively (National Audit Office, 2001). By 2007, these costs had increased to £4.2 billion and £15.8 billion respectively and the financial burden of obesity is projected to increase further over the next decade (Butland et al. 2007).

1.1.2. Causes of Obesity

Obesity was classified recently as a pathophysiological disease by the American Medical Association (AMA 2013). It is widely accepted that that obesity is caused by an energy imbalance whereby there is greater energy intake than energy expenditure (WHO, 1998). This could be due to an increase in energy intake, a decrease in energy expenditure, or both. However, the causes of this imbalance are complex. Some evidence suggests that overall energy expenditure has not declined in recent decades in Europe and the United States (Westerterp and Speakman, 2008). However, other data suggests that energy expenditure at work has decreased in the United States over the last 50 years (Church et al. 2011). It should be noted though that these data do not cover non-occupational energy expenditure, such as exercise or recreational physical activities. Thus, on balance, it seems more likely that an increase in energy intake may underlie the increase in obesity. Indeed, Swinburn and colleagues (2009) reported that the increase in energy intake over the last few decades, best explains the obesity epidemic in the United States.

One theory that addresses how energy intake has increased asserts that the environment of the developed world provides ready access to energy dense foods, that promotes over indulgence; the so called 'obesogenic environment' (Swinburn et al. 1999). In addition to the ready availability of energy dense food, it has been reported that meal portion sizes have increased in parallel to increasing body weight in the US, suggesting that this phenomenon might also be responsible for the increased energy intake (Young and Nestle, 2002). More recent work has also suggested that eating occasions have increased, so that individuals are now eating more meals and snacks which may be contributing to overall increases in energy intake (Duffey and Popkin, 2011). Hence, readily available food, increased portion size, and frequency of consumption are all important factors related to increased energy intake. However, not all individuals exposed to an obesogenic environment become obese, therefore there must be other factors involved.

Genetics are one such factor. It has been reported that identical twins reared apart show similar body weights (Stunkard et al. 1990), and that when identical twins are overfed over a long period, they show similar weight gain (Bouchard et al. 1990). Genetic factors explain up to 70% of BMI variance in the general population (Comuzzie and Allison, 1998), and this increases to a maximum of 90% in twins reared apart (Maes et al. 1997). While this strongly suggests that genetics are involved in weight (and weight-gain) the question arises; how do genetics contribute to obesity?

It is possible that the genes involved mediate reward responses to food stimuli. Obese individuals show enhanced reward responses to food (Carnell, 2012), which are predictive of future weight gain (Yokum et al. 2011; Demos et al. 2012). The hormones leptin and ghrelin

regulate food reward (Farooqi et al. 2007; Malik et al. 2008), and are associated with the fat mass and obesity-associated gene (FTO) (Wang et al. 2011; Karra et al. 2013). In addition, genetic polymorphisms of dopamine receptors, have been associated with differential brain responses to palatable foods, which are predictive of increases in body mass (Stice et al. 2010). Hence, there is compelling evidence linking genetics to food reward and weight gain.

One issue to be resolved is why we would possess a genetic susceptibility for weight gain, and why this might be more an issue now than previously. The leading theory which addresses this point is known as the "drifty gene" hypothesis (Speakman, 2008). It proposes that genes which control the upper limit on body weight have randomly drifted, resulting in a larger number of individuals predisposed to gain weight. Hence, it seems possible that some of the genes that have 'drifted' might be those that underlie reward responsiveness to food, thereby promoting the overconsumption of energy dense palatable foods, resulting in obesity (Speakman et al. 2011).

The involvement of both environmental and genetic factors suggests the two may interact whereby a genetic susceptibility to obesity is more likely to be expressed in an obesogenic environment. Previous models relying exclusively on genetic and biological explanations or social and environmental explanations of obesity have been criticised for ignoring gene-environmental interactions, leading to newer models that account for these, that better fit the available evidence (Speakman et al. 2011).

In terms of targeting genes and environment, genetic treatments for obesity are unlikely to appear in the near future and it is unknown whether such treatments will be effective or free

from side effects. In addition, redesigning the global food environment is neither a simple or swift task. However, as neurotransmitter and neurohormonal systems underpin these genetic-environmental interactions, the most sensible immediate strategy for treatment would be to focus on the neurocircuitry that underlies responses to food via centrally acting anti-obesity drugs.

1.1.3. Summary

Obesity is a global problem and it's worldwide prevalence is associated with severe health and economic consequences. Increased energy intake due to easily accessible food, larger portion sizes and increased eating frequency appear to be key contributors to weight gain. However, genetic influences are likely to interact with the environment, such that individuals are more likely to over-consume in the current food environment due to genetic predisposition. Treatments are urgently needed for obesity and to curb enhanced energy intake.

1.2. Appetite and Food Intake

1.2.1. Introduction

As the over-consumption of food is a major factor in obesity, a clear understanding of human appetite and eating behaviour is necessary if new approaches to obesity treatment are to be developed. Ingestion of food provides a flow of nutrients to meet immediate energy needs. Energy can also be stored within the body to be used when circulating energy levels drop. Such stores exist within the liver and within fat cells, and accumulation of the latter is particularly problematic in obesity (Hill et al. 2012).

Although the initiation and termination of food intake is primarily influenced by metabolic state (i.e. eating is initiated by food deprivation and terminated by eating) there are additional influential factors. For instance, enhancing palatability can increase food consumption in the absence of physiological hunger (Fisher and Birch, 2002). More food may be also consumed at breakfast than usual if an individual is likely to miss lunch. Conversely, an individual might stop eating when hungry if they have a dietary goal and are actively restricting the amount they consume (Herman and Polivy, 1984; Boon et al. 1998; Johnson et al. 2012). Social circumstances can also influence eating and affect food intake such that people eat more if other diners are eating more and eat less if others eat a small amount (Herman et al. 2003). Hence, the consumption of food is influenced by a range of factors in addition to metabolic state, as indicated in Figure 1.1 (below).

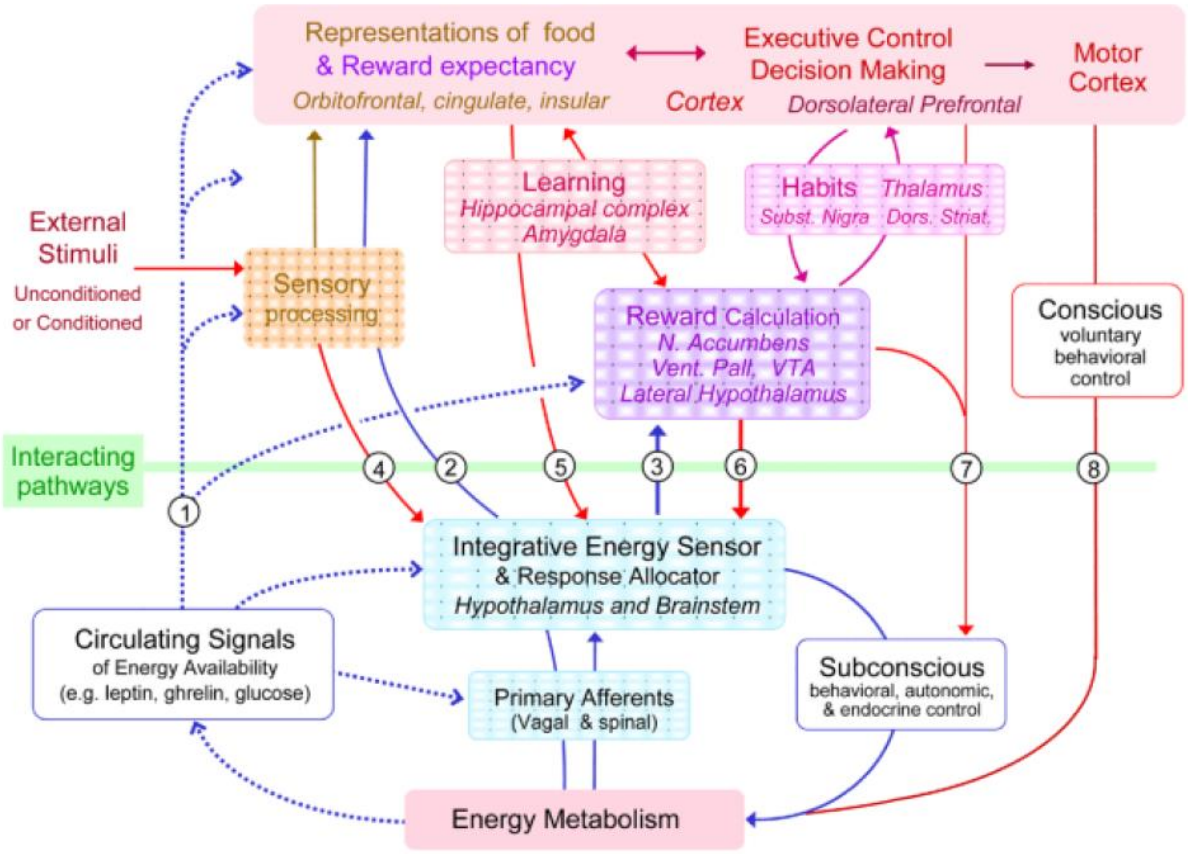

Figure 1.1. The control of appetite (replicated directly from Berthoud, 2011)

"Schematic diagram showing potential interactions between the so called "homeostatic" energy balance regulatory system (blue) and neural systems involved in external sensory information processing (yellowish-brown), reward processing (purple), and cognition and executive functions (red), collectively referred to as "hedonic systems". Blue arrows indicate bottom-up modulation of hedonic systems by homeostatic signals. Broken blue lines represent circulating hormones, metabolites, and other factors; solid blue lines represent neural pathways. Red arrows indicate top-down modulation of homeostatic processes by hedonic drives."

Eating behaviour is orchestrated by a manifold system of neural circuitry, comprised of several distinct but interacting components (Figure 1.1). This includes circuitry governing: the sensory processing of appetitive stimuli, energy balance, food reward and cognitive mechanisms involved in appetite. Each of these systems will now be reviewed in turn.

1.2.2. Gustatory and Sensory Processing of Appetitive Stimuli

The consumption of food involves the stimulation of a variety of sensory pathways. These include: gustatory, oral, visual and olfactory pathways. Visceral axons from the gut and orosensory neurones from the mouth both project to the medulla oblongata at the nucleus of the solitary tract (NTS) (Altschuler et al.1989; Travers and Norgren, 1995). Hence, the NTS is considered one of the first major 'stations' in the neuraxis for the convergence and processing of visceral and oral sensory input (Grill and Hayes, 2009). The NTS projects to the insula which is also known as the primary taste cortex (Saper et al. 1980).

Early work by Penfield and Faulk (1955) noted that electrical stimulation of the insula in humans produced visceral sensory phenomena such as nausea and gurgling, oral phenomena such as chewing and swallowing, and sensations of taste or smell. More recent work with primates has confirmed that the insula is involved in encoding taste and other oral somatosensory stimuli allowing 'higher' cognitive prefrontal structures to utilise this information in the execution of decisions which lead to behaviour (Rolls, 2006; Yaxley et al. 1990; Verhagen et al. 2004). Hence, sensory encoding of food stimuli in the insula is conveyed to the adjacent orbitofrontal cortex (OFC) (Shi and Cassell, 1998; Vertes, 2004).

Visual and olfactory information about food is also relayed to the OFC. The initial processing of visual information occurs in parts of the cortex specialised for visual stimuli (visual areas 1, 2 and 4; V1, V2, and V4), before being processing by the inferior temporal visual cortex and being relayed to the amygdala and the OFC (Rolls, 2007). In addition, stimulation of the olfaction sensory system by the smell of food via chemoreceptors in the nose involves

activating the olfactory bulb. This then projects this sensory information to the olfactory pyriform cortex, and then as for visual stimuli, to the amygdala and OFC (Rolls, 2007).

Hence, the majority of sensory pathways responsible for processing food stimuli converge at the OFC. The presence of multimodal neurones capable of coding two sensory stimuli simultaneously (e.g. taste and olfaction) in the OFC has led to the theory that it is the first area of cortex where modalities converge and are represented as a combined whole (Rolls and Bayliss, 1994). Evidence suggests that the resulting multi-modal sensory representation is projected to the hypothalamus, where it is integrated with metabolic signals to influence the consumption of food (Rolls, 2000).

1.2.3. Energy Balance Circuitry

Appetite is strongly influenced by an individual's energy balance state, such that the ingestion of food is more likely when an individual requires energy and is 'hungry' than when they do not require energy, such as when they are 'full' after eating food (Zheng et al. 2009). The neural circuitry that monitors this state of energy balance has been intensively researched, and includes brainstem structures such as the NTS along with the core 'homeostatic' area, the hypothalamus (Berthoud, 2002).

As noted earlier the NTS receives direct gustatory input, making it the earliest structure to receive information that might signal a change in energy state (i.e. incoming energy). The NTS contains fenestrated capillaries that allow peripheral blood-based factors such as hormones to modulate neural activity at this site (Cunningham, 1994). This is an important element of energy balance, as there are a number of hormones that interact with neural

circuitry to communicate energy state. For instance, ghrelin is produced in the gastrointestinal tract and is secreted when the stomach is empty, while leptin is produced by fat cells as an indicator of fat stores (Klok et al. 2007). Hence, ghrelin serves as a more immediate indicator of energy needs while leptin acts as a longer term regulator of energy stores (Klok et al. 2007).

Recent evidence shows that infusion of leptin into the medial NTS reduces food intake in rats (Kanoski et al. 2014), while an anti-ghrelin antibody-induced reduction in NTS ghrelin achieves the same effect (Solomon et al. 2006). Hence, leptin and ghrelin are referred to as satiety and hunger hormones, respectively. Cholecystokinin (CCK) and Glucagon-like peptide-1 (GLP-1) are also produced and released in the gastrointestinal (GI) tract upon detection of relevant incoming nutrients, and act at receptors on neurones in the NTS to signal nutritive content (Glatzle et al. 2001; Punjabi et al. 2011). Consequently, the NTS receives multiple neuro-hormonal signals indicating current and long term energy balance, which the NTS projects onwards to the hypothalamus (Beckstead et al. 1980).

The hypothalamus includes the dorsomedial hypothalamus (DMH), ventromedial hypothalamus (VMH), lateral hypothalamus (LH), arcuate nucleus (ARC) and the paraventricular nucleus (PVN). Through the use of electrolytic and neurotoxin lesion and drug infusion studies in rodents, the roles of these hypothalamic sub-structures in appetite and food intake have been elucidated. For instance, lesions of the VMH and PVN lead to hyperphagia (Hetherington and Ranson, 1942; Anand and Brobeck, 1951; Shor-Posner et al., 1986). Conversely, early LH lesion studies induced hypophagia (Anand and Brobeck, 1951; Teitelbaum and Epstein, 1962). However, the selectivity of early LH lesions has been

questioned. For instance, Ungerstedt (1971) performed chemical lesions of the LH using 6-hydroxydopamine (6-OH-DA), which successfully induced hypophagia, but also destroyed axons contained in the medial forebrain bundle (MFB), which provide most of the monoaminergic innervation of the forebrain. Thus, the lesions caused severe deficits in locomotion and the rats were unable to move and so unable to eat and drink unless tube fed. However, subsequent work with LH lesions produced with kainic acid which were more selective and spared MFB fibres, induced hypophagia in the absence of sensory-motor deficits (Grossman et al. 1978).

One of the most important nuclei in the hypothalamus is the ARC nucleus. The ARC integrates signals from the NTS (Heijboer et al. 2006). It is also accessible to large peptide molecules due to its proximity to a section of the blood-brain barrier that is semi-permeable (Cone et al. 2001). ARC neurones are referred to as 'first-order' neurones due to their direct contact with peripheral peptide hormones, whereas neurones in the PVN, LH, VMH, and DMH are referred to as 'second-order' neurones (Heijboer et al. 2006). Peptides within the ARC nucleus are crucial in the regulation of energy balance and control of food intake. Activation of first order neurones that coexpress agouti-related peptide (AgRP) and neuropeptide Y (NPY) stimulates food intake in rats, whereas activation of neurones expressing pro-opiomelanocortin (POMC) inhibits food intake in rats (Hahn et al, 1998; Elias et al. 1999; Cowley et al. 2001).

POMC neurones in the ARC nucleus might be particularly crucial to energy balance and could represent a potential target for anti-obesity drugs. Serotonin (5-hydroxytryptamine) is a key neurotransmitter involved in the control of appetite (Blundell, 1977; Burton et al. 1981; Clifton et al. 1989; Blundell, 1986). Serotonin is implicated in the hypothalamic control of

food intake. For example, serotonergic agonists binding at receptors in the hypothalamus reduce food consumption in rats (Shor-Posner et al. 1986; Dourish and Hutson, 1989; Leibowitz and Alexander, 1998; Smith et al. 1999). Recent evidence indicates that enhancement of serotonergic function in the ARC suppresses appetite via effects on POMC neurones, suggesting that POMC receptors could be crucial downstream targets for future weight-loss drugs (Sohn et al. 2011; Heisler et al. 2002).

Dopamine and noradrenaline acting in the hypothalamus also affect food intake. Infusion of either dopamine, the dopamine precursor L-Dopa, or the dopamine agonist d-amphetamine into the LH decreases food intake in rats (Leibowitz and Rossakis, 1979). In addition, infusion of the selective D_2 dopamine receptor antagonist sulpiride into the LH increases food intake in rats (Sato et al. 2001). In contrast, infusion of noradrenaline into the LH and the PVN increases food intake in rats and pigeons (Leibowitz, 1978; Hagemann et al. 1998). It is probable that many anti-obesity drugs achieve their weight-loss effects, at least in part, by acting in the hypothalamus to alter energy balance signals. However, targeting this system may be less successful in reducing eating that is motivated by the hedonic appeal of foods.

1.2.4. Food Reward

While energy state clearly plays a role in eating behaviour, the consumption of food is not singularly under the control of energy balance circuitry. It is well documented that highly palatable foods are eaten in greater amounts than foods that are less palatable (Yeomans, 1996). In addition, evidence shows that as foods are eaten their rated pleasantness declines when compared to a food that has not been eaten, bringing about meal termination (Rolls et al. 1981). This process is referred to as sensory specific satiety (SSS; Rolls et al. 1981).

Hence, the pleasure, or 'hedonic' aspects of eating food also influence appetite and food intake.

It has been suggested that dopamine mediates the subjective pleasure associated with eating food (Wise, 1978; Wise, 2008). However, in recent years this idea has been challenged by evidence that reward consists of both 'liking' (hedonic pleasure) and 'wanting' (motivation to acquire a stimulus) and that dopamine is more closely associated with incentive salience (the 'wanting' of stimuli), rather than 'liking (Berridge, 2007). The distinction between wanting and liking is crucial to understand conventional reward, which involves both of these processes. For instance, liking a stimulus leads to a positive affective state, but without wanting, does not lead to acquisition of the rewarding stimulus (i.e. there is no motivation to acquire the stimulus). Conversely, wanting without liking will lead to the acquisition of a stimulus, but in the absence of pleasure (no hedonic value). Hence, both wanting and liking are needed for reward.

The distinction between liking and wanting can be observed on both a neural and behavioural level. There is evidence that a limited number of anatomically small brain regions are involved in liking, whereas a larger network of regions is involved in wanting (see Berridge et al. 2010). Behaviourally, the pleasure (liking) elicited by a stimulus can be measured in rodents by examining orofacial responses and body movements. Typically, sweet pleasurable tastes elicit positive orofacial responses (tongue protrusions, etc.), and can be observed in the absence of dopamine-mediated wanting (Berridge et al. 1998). 'Wanting' is measured as a function of motivation to acquire a stimulus. It can be measured in various ways, such as via the latency to obtain food and can be dissociated from 'liking' (Peciña et al. 2003). Hence,

food reward in the contemporary sense can be conceptualised as the combination of food "wanting" and "liking". The pharmacological mechanisms of liking and wanting are complex, and extend beyond dopamine mechanisms. For instance, opiodergic mechanisms have been reported to mediate liking in humans (Yeomans and Gray, 1996) and also food "wanting" (for review see Berridge, 2009). Further exploration of wanting and liking will be discussed below.

1.2.4.1. 'Wanting'

The neural substrate of wanting, or incentive salience, comprises a number of brain regions. The ventral tegmental area (VTA) is one of the first brain areas in the motivational neuraxis, wherein a crude representation of stimulus motivational relevance is formed. It is then projected onwards to the ventral pallidum, the nucleus accumbens (NAcc), amygdala, hippocampus and prefrontal cortex (Fields et al. 2007). Many of these areas form the basis of the mesolimbic and mesocortical dopamine pathways, which in turn, relay the motivational relevance of food stimuli to additional brain areas involved in the wider appetite circuitry.

A key brain area in both of these pathways is NAcc, which comprises two regions: the core and the shell (Kelley et al, 2005). Experimental evidence from rodents suggests that the core and shell are innervated by the dopaminergic nigrostriatal and mesolimbic pathways, respectively (Deutch and Cameron, 1992). These are involved in the control of motivation and motor actions (Drui et al. 2014; Kim et al. 2003), and the reinforcing effects of stimuli such as drugs and food (Martel and Fantino. 1996; Pierce and Kumaresan, 2006). Hence, there is strong evidence that both the core and shell of the NAcc are involved in wanting behaviours.

Other striatal structures are also involved in food motivation, such as the caudate and putamen, collectively referred to as the dorsal striatum. Injection of a dopamine antagonist into the dorsal striatum decreases lever pressing for food in rats (Beninger et al. 1993). In addition, DiFeliceantonio and colleagues (2012) found that injection of a μ-opioid receptor agonist into the rat dorsal striatum dramatically increased intake of a palatable sweet food by 250%, without changing the hedonic impact of sweet tastes, as measured by a taste reactivity test. Hence, wanting can be dissociated from liking in rats (DiFeliceantonio et al. 2012).

The amygdala is also involved in motivation and can be divided into several nuclei, including the central nucleus, medial nucleus, cortical nucleus, and basolateral group (Solano-Castiella 2010). Recent work suggests that the amygdala stores associations between conditioned stimuli and motivational and affective values (Fernando et al. 2013. It has also been suggested that the central nucleus encodes motivational value, while the basolateral amygdala encodes emotional events with reference to sensory-specific aspects (Balleine and Killcross et al. 2006). Lesions of the basolateral amygdala interfere with the representation of sensory aspects of motivational events but not neutral events (Dwyer and Killcross, 2006), suggesting that the amygdala provides an input for sensory information into the motivational pathway. The amygdala also relays motivational information to the OFC where it is used in the selection and execution of subsequent eating behaviour (Schoenbaum et al. 1999).

1.2.4.2. 'Liking'

Liking is associated with a number of brain regions including sites in the brainstem through to the prefrontal cortex. It is thought that the 'liking response' to food is mediated at least in part by "hedonic hotspots" in the brain. These are cubic-millimetre regions of neurones which enable opioid and endocannabinoid amplification of sensory pleasure (e.g. liking of food)

(Berridge, 2009). Such hotspots have been identified in rodents in the NAcc and ventral pallidum, are indicated to exist in the brainstem (parabrachial nucleus) and may also exist in the amygdala and OFC (Berridge, 2009; Castro and Berridge, 2014a). Hedonic hotspots are part of a distributed network across the brain, forming an integrated liking system.

Initial liking reactions to food stimuli are generated in the brainstem. Decerebrate rats that have the brainstem isolated from the forebrain are capable of generating orofacial liking reactions to sweet tastes (Grill and Norgren, 1978). Opioid mechanisms are thought to mediate these liking reactions. Thus, opioid receptor agonists and antagonists infused into the NTS increase and decrease respectively food intake in rats (Kotz et al. 1997; Kotz et al. 2000) and modulate feeding by acting on the rat parabrachial nucleus (Carr et al. 1991). Multiple opioid receptor subtypes are implicated in liking and food intake, although μ-opioid receptors are thought to play the most important role (Gosnell et al, 1986). In addition to opioids, injection of gamma-aminobutyric acid (GABA) into the rostral-medial accumbens enhances eating behaviour and liking reactions in rodents (Reynolds and Berridge, 2002). Also, benzodiazepine injection into the parabrachial nucleus enhances food intake (Higgs and Cooper, 1996) while injection into the brainstem of decerebrate rats enhances liking reactions (Berridge, 1988). Hence, multiple neurotransmitters mediate liking responses.

The NTS projects onwards to the NAcc (Brog et al. 1993) which contains a hedonic hotspot, a small region in the NAcc medial shell (Peciña and Berridge, 2000). In a study with rats, orofacial liking reactions to sucrose were enhanced by opioid stimulation of the rostromedial shell of the NAcc (Peciña and Berridge, 2005). In addition, it has been reported that infusion of the endocannabinoid anandamide into the medial shell hotspot induced enhancement of liking reactions to sucrose in rats (Mahler et al. 2007), suggesting involvement of

endocannabinoid mechanisms. The involvement of cannabinoid and opioid mechanisms is also supported by reports that delta(9)-tetrahydrocannabinol and anandamide enhance sucrose palatability in rats (Higgs et al, 2003) and that opioids are strongly associated with the hedonic perception of food stimuli (Berridge, 1996; Barbano et al. 2007). There is also recent evidence for a negative hedonic coldspot in the caudal half of the medial shell, where opioid stimulation suppresses liking reactions (Castro and Berridge, 2014b).

The NAcc projects onwards to the prefrontal cortex (PFC) (Berendse et al. 1992), which has also been implicated in liking responses. Two subdivisions that have been the focus of research include the OFC and the ventromedial PFC (vmPFC). In humans, BOLD activity in the OFC correlates with the rated pleasantness of appetitive stimuli, leading to the conclusion that brain activity in this area represents the pleasure or 'liking' associated with food (Kringelbach et al, 2003). The adjacent vmPFC has also been implicated in liking, and in an fMRI study with healthy volunteers across a wide age range, increased BOLD activity in the vmPFC was associated with more positive valence ratings (Winecoff et al. 2013). In addition, greater BOLD responses were detected in the vmPFC to sucrose when it was rated as pleasant by participants (Rudenga and Small, 2013).

While there appears to be a functional overlap between the OFC and vmPFC, a recent study with monkeys (Bouret et al. 2010) provided evidence that while both areas are responsive to liking stimuli, OFC neurones are more sensitive to external/environmental stimuli such as visual cues, while vmPFC neurones are more sensitive to internal/subject specific stimuli such as satiety. This is supported by evidence showing strong connections between sensory cortical areas and the OFC (Ongur and Price, 2000), and the observation that vmPFC activity

strongly correlates with internal goal values in humans (Hare et al. 2009). Together, these prefrontal liking evaluations guide food reward, learning, and decision making (Rushworth et al. 2011).

There is an extensive body of research using measures of liking and wanting in humans. However, it is unclear whether these studies have measured the same constructs that have been investigated in rats. For instance, Volkow and colleagues (2002) have reported that in humans, increases of extracellular dopamine in dorsal striatum are associated with increased ratings of desire to eat food. However, Small and colleagues (2003) found that dopamine levels in the dorsal striatum correlated with meal pleasantness, but not desire to eat, or satiety (Small et al. 2003) suggesting that dopaminergic effects are associated with pleasure, but not motivation in humans. More recently, there has been a debate over whether wanting and liking can be properly measured and dissociated in humans at all (see the following for the most recent discussion: Havermans, 2011; Finlayson and Dalton, 2012; Havermans, 2012). Although this is an ongoing debate, it seems unlikely that present methods successfully measure in humans the type of wanting and liking observed in rats, for instance, free from social cognitions, etc. (Havermans, 2012).

1.2.5. Interactions between Energy Balance and Reward Circuitry

Energy balance and reward have been discussed separately, but these neural circuits do not function independently. Indeed, the hypothalamus is reciprocally connected to and influences, or is influenced by, multiple reward regions (Kelley et al. 2005). For instance, the NAcc shell projects to several hypothalamic sites, including the ARC and LH (Baldo et al. 2004) to influence feeding in rats (Kelley et al. 2005). However, the LH also projects back to

the NAcc shell to modulate reward, and consequently, food intake (Sears et al. 2010). In addition, leptin modulates the activity of dopamine neurones in the ventral tegmental area, which project to the nucleus accumbens; enabling metabolic signals to modulate reward (Fulton et al. 2006). Hence, energy balance can influence reward processing, and vice versa, such that both the hypothalamus and NAcc jointly co-ordinate reward-satiety processes.

Other limbic regions involved in reward, are also connected to the hypothalamus. There are neuronal pathways between the amygdala and the VMH, damage to which causes hyperphagia and obesity in rats (King, 2006). Serotonin mediates some of these amygdala-hypothalamic effects. Administration of the selective serotonin reuptake inhibitor zimelidine into the medial nucleus of the rat amygdala produces a hypophagic effect, which is blocked by the selective 5-HT_{2C} receptor antagonist SB-242084 (Scopinho et al. 2012). It is possible that this serotonin mechanism could be upstream of melanocortin expressing neurones in the amygdala that also affect appetite for dietary fat (Boghossian et al. 2010). Moving along the neuraxis, the PFC is also connected to the hypothalamus (Rempel-Clower and Barbas, 1998). It has been suggested that the OFC is sensitive to energy balance information (Rolls et al. 1989) because activity in the OFC decreases as participants become satiated (Kringelbach et al, 2003). Hence, both energy balance and reward circuitry across the neuraxis interacts in an adaptive manner to co-ordinate eating behaviour.

1.2.6. Cognitive Mechanisms Involved in Appetite

Cognitive mechanisms also play an important role in mediating human eating behaviour. At the most basic level, attentional mechanisms are needed to orientate individuals towards food stimuli. Executive resources are required to make decisions, such as whether to approach, or

inhibit the approach towards food. Memory is also required to store associations and outcomes with food and for learning to take place.

A key brain region implicated in cognitive control is the cingulate cortex, which can be split into anterior (ACC) and posterior (PCC) divisions. It is thought that the PCC is involved in sensory events and plays a role in spatial orientation and memory, while the ACC is involved in executive tasks, which involve emotional control of visceral related functions (Vogt et al. 1992). More recent evidence suggests that there may be a further subdivision of function within the ACC, whereby the dorsal region is involved in cognitive control and the rostral ACC is more engaged with emotion regulation in humans (Mohanty et al, 2007). Weissman and colleagues (2004) have reported that the dorsal ACC focuses attention on behaviourally relevant stimuli and Holroyd and Yeung (2012) have suggested that the ACC selects and maintains behaviour directed towards salient goals. Thus, lesions of the ACC in rats produces a marked decrease in foraging for food, but not in other behaviours (Li et al, 2012) and Rhesus monkeys with ACC lesions show impairments in using rewarded trials to sustain selection of an appropriate object during a learning task (Chudasama et al. 2012).

Furthermore, antagonism of D_1 dopamine receptors in the ACC reduces preference for a high-cost high-reward response option in rats, suggesting that D_1 dopamine receptors are involved in ACC regulation of motivation-based decisions (Schweimer and Hauber, 2006). In addition, ACC opioid blockade reduces ethanol seeking behaviour in mice suggesting that opioidergic neurotransmission is involved in maintaining a cue conditioned reward signal (Gremel et al. 2011). Hence, the ACC is probably an important area for maintaining goal-directed behaviours.

Cognitive processes may inhibit as well as enhance approach to food. For instance, increased tonic activity in the dorsolateral prefrontal cortex (dlPFC) has also been associated with greater behavioural inhibition (Shackman et al. 2009) and a recent meta-analysis reported reduced dlPFC activation to food images in obese versus lean participants (Brooks et al. 2013a) suggesting a role for inhibiting appetitive behaviours. The vmPFC is functionally connected to the dlPFC, and the dlPFC shows increased activity when individuals exercise self-control (Hare et al. 2009). Further, dlPFC activity modulated vmPFC signal in response to food, suggesting that inhibitory control can modulate reward signalling in humans (Hare et al. 2009). It is likely that inhibitory control is achieved via the input of other cognitive functions, as the dlPFC is involved in preparing for action, based on information stored in short-term memory (Pochon et al. 2001).

The hippocampus is crucial for the control of learning and memory (Scovile and Milner, 1957; Eichenbaum and Cohen, 1993) and projects to areas including the hypothalamus, NAcc, amygdala and PFC (Van Groen and Wyss, 1990; Barbas and Blatt, 1995). Studies in rats, monkeys and humans, using neurophysiological, neuropsychological and neuroimaging techniques, suggests that the hippocampus plays a pivotal role in memory recall (Eichenbaum et al. 2007). Recent evidence suggests that the hippocampus is involved in the control of food intake (Davidson et al, 2007) as hippocampal lesions in rats increase energy intake and weight gain (Davidson et al. 2009). In humans, similar effects have been found with amnesic patients with hippocampal damage, who consume multiple meals (consuming more food than controls), in the absence of changes in rated appetite (Higgs et al. 2008). Further studies with healthy volunteers have reported that disrupting the formation of episodic memories of meal consumption, subsequently leads to increased food intake (Higgs and Donohoe, 2011). Hence, memories of food, and of food consumption, represent an important cognitive

mechanism of eating behaviour and a potential target to exploit in future weight-loss treatments.

1.2.7. Summary

The regulation of appetite and food intake is complex. Sensory engagement with food stimuli engages multiple neural sites, from the brainstem where initial processing of gustatory stimuli begins, to the prefrontal cortex, where multimodal representations of sensory input are formed. At the same time, circuits involving the brainstem and hypothalamus co-ordinate energy sensing and responses to peripheral hormonal indicators of short and long-term energy balance. Interacting with these systems, reward circuitry induces motivation for relevant appetitive stimuli (wanting), and generates pleasure (liking) reactions to food. Finally, cognitive mechanisms including attention, executive control and memory are important for control of eating behaviour. Together, the interaction of these neural circuits creates a complex, dynamic and adaptive eating behaviour control system. Given that this circuitry is dependent on neurotransmitter function, a tempting prospect for treating obesity is to target these systems with anti-obesity drugs. For instance, drugs might be designed to: (a) influence motivational centres, to reduce the 'wanting' of food; (b) influence pleasure related areas to reduce the impact of highly palatable foods; (c) influence energy sensing circuitry to reduce hunger or enhance satiety; (d) influence cognitive functions such as memory, to enhance memories of food consumption and reduce subsequent food intake. These are only a few examples, but they illustrate how the control of appetite can be modulated in multiple ways, at multiple sites, via multiple neurotransmitter systems.

1. 3. Treatment of Obesity

1.3.1. Introduction

The primary aim of obesity treatment is weight-loss. Even a modest 5-10% weight-loss, which is approximately 5kg-10kg in patients with a healthy BMI, is associated with improvements in health (Goldstein, 1992; Blackburn, 1995). Weight-loss in excess of 10%, which is > 10kg weight-loss in those with healthy BMIs, is classed as moderate and shows superior improvements in health, such as decreased blood pressure and cholesterol and improved glycemic control (Pasanisi et al. 2001; Wing et al. 2011). Current methods of treating obesity include lifestyle interventions, psychological therapies, bariatric surgery and anti-obesity drugs.

1.3.2. Lifestyle Interventions

One of the most popular lifestyle interventions is the use of diets, of which there are a staggering variety. For instance, there is a subset of diets that focus on varying the intake of certain types of foods. Low fat diets advocate reducing the amount of fat consumed and show a mean weight-loss of 3.2kg after at least 2 months of intervention (Astrup et al. 2000), while low carbohydrate diets decrease weight by 7.0kg after 3 months intervention (Santos et al. 2012). Conjunction diets, such as low carbohydrate – high protein can also reduce weight to a greater extent that singular diets (e.g. high protein only) (Hession et al. 2008). While some individuals follow these diets alone, many join dieting groups. For instance, commercial weight-loss programs such as Weight Watchers provide dietary advice and social support and produce modest weight-loss of approximately 4.4 - 5.1kg after 12 months (Jolly et al. 2011; Jebb et al. 2011).

In addition to diets, physical activity is often prescribed as a weight-loss adjunct, although there has been considerable debate regarding whether exercise may enhance energy intake and thus have a counterproductive effect. For example, there is evidence that exercise is associated with increased energy intake (Sonneville and Gortmaker, 2008) and that individuals compensate for up to 30% of an exercise-induced energy deficit by enhancing their energy intake (Stubbs et al. 2004; Whybrow et al. 2008). In contrast, increased exercise does not increase hunger, or energy intake (King et al. 1997) and a recent systematic review of 103 research studies found no evidence of increased energy intake following increased exercise (Donnelly et al. 2014). Since obese individuals do not tend to show these compensatory effects (Donnelly et al. 2000) but lean individuals do (Woo and Pi-Sunyer, 1985), it has been proposed that the compensatory effects are specific to lean individuals, as a mechanism to defend their fat reserves (King et al. 2012).

In a weight-loss intervention study in the obese, promoting higher levels of physical activity led to greater weight-loss after 12 and 18 months, compared to controls given standard behaviour therapy (Jeffrey et al. 2003). Similarly, exercise has been proposed to be at least as effective as dietary interventions, with evidence that modest weight-loss of 7.6kg can be achieved after 3 months of either a low calorie diet, or increased exercise in obese men (Ross et al. 2000). However, not all studies report such large effects and a meta-analysis by Miller and colleagues (1997) showed that 4 months of exercise only resulted in a 2.9kg weight-loss.

While the degree of weight-loss varies, the majority of research concurs that the combination of diet and exercise intervention provides greater weight-loss in the long term, than either intervention used in isolation. For instance, in the same meta-analysis by Miller and

colleagues (1997), diet produced a moderate 10.7kg decrease in weight, while diet and exercise decreased weight by 11.0kg. However, after a 1 year follow-up, maintenance of weight-loss effects was 6.6kg for the diet and 8.6kg for the diet and exercise group, suggesting improved maintenance effects of combining exercise with diet (Miller et al. 1997). A more recent systematic review has also reported that exercise in conjunction with diet, results in greater weight-loss than diet alone (Curioni and Lourenço, 2005) and that these benefits can persist for over 2 years (Wu et al. 2009).

A limitation of the diet and exercise approach is that maintenance of weight-loss effects is often poor. In many cases almost half of initial weight-loss is regained 12 months after either diet, or diet and exercise interventions (Curioni and Lourenço, 2005). According to a systematic review of the literature, while diet and exercise may produce a moderate weight-loss of 11.1kg after four months, weight-loss maintenance drops to 5.8kg after 1 year (Kouvelioti et al. 2014). One explanation of weight regain is that there are biological compensatory mechanisms which alter behaviour to 'correct for' weight-loss (Leibel et al. 1995; see Blomain et al. 2013 for review). However, this is a generic hypothesis that applies equally to all mechanisms of weight-loss. Specifically in relation to dieting, food liking is enhanced after calorie restriction interventions, suggesting that in the long term, dieting might increase the reward value of food, leading to over-consumption that promotes weight re-gain (Cameron et al. 2008). With regard to exercise, obesity is associated with reduced pleasure taking exercise, particularly due to social anxiety, which might explain poor long-term compliance and subsequent weight regain (Ekkekakis et al. 2010).

Weight cycling is the cyclical losing and gaining of weight between diets (Blackburn et al. 1989). In a population study of almost 7000 people approximately 7% of men and 10% of women were severe weight cyclers, with weight loss more than or equal to 5kg on three

occasions accompanied by weight regain (Lahti-Koski et al. 2005). There is evidence that it is associated with increases in binge eating (Foster et al. 1997) and is a predictor of large weight gain (Kroke et al. 2002). Hence, gaining and losing weight via successive weight-loss interventions such as dietary and exercise interventions might have a net negative effect on weight and health. However, it should be noted that not all studies have found such effects (Stevens et al. 2012).

Both diet and exercise are capable of producing modest to moderate weight-loss. A combination of diet and exercise seems to be the most efficacious approach, but maintenance of weight-loss is an ongoing problem. Recurrent weight cycling due to repeated dieting attempts is also common and has negative consequences. Thus, neither dietary nor exercise interventions are effective for sustaining moderate (i.e. 10% or more) decreases in body weight.

1.3.3. Psychological Therapy

There are a variety of psychological therapies aimed at supporting lifestyle changes. Cognitive behavioural therapy (CBT) is one of the leading therapies. Cooper and Fairburn (2001) proposed the idea of adapting CBT to the treatment of obesity, based on the premise that changing thinking related to eating behaviour and weight would result in a positive effect over time. The aim of CBT for obesity is to adjust an individual's thinking about their weight and weight-loss attempt(s) to help them achieve their goals.

It has been reported that eight sessions of CBT compared to being on a waiting list, resulted in a small but statistically significant reduction in weight in adult women, which was sustained at a 4 month follow-up (Pimenta et al. 2012). In adolescents, 20 weeks of CBT

compared to a no treatment control group resulted in reductions in body weight (-1.9kg) and body fat (-1.5kg) and reduced the intake of sugary drinks which was directly related to the reductions in weight (Tsiros et al. 2007). In another study comparing CBT programmes ranging from 14-20 weeks in length, approximately 6% of body weight was lost during the interventions. However, compliance and follow-up attendance were poor (Melchionda et al. 2003).

Typically, CBT is not administered alone, but as an adjunct therapy. A combination of CBT, diet and exercise was found to produce greater reductions in weight than diet and exercise alone (Shaw et al. 2005). However, for both CBT delivered alone and CBT administered in combination with other interventions, the evidence suggests that while it is possible to induce weight-loss, the effect is unlikely to be maintained. In a five year follow-up study, morbidly obese patients were given a comprehensive intervention, comprising CBT, diet and exercise, during a 6 week stay in hospital (Golay et al. 2004). Although modest weight-loss of 7.6kg was achieved, after 5 years more than half of patients had regained this weight (mean weight regain = 10.4kg). In another study, obese patients were given CBT compared to guided self-help (minimal intervention). Both interventions resulted in approximately 10% weight-loss, however, after 3 years follow-up, both groups regained almost all the weight they had lost (Cooper et al. 2010). Hence, while CBT might represent a non-invasive way to induce weight-loss, it lacks the ability to produce sustained weight-loss.

Mindfulness training is a type of psychological therapy that has been trialled to assist with weight-loss and weight-loss maintenance. Mindfulness raises awareness of an individual's awareness of thoughts and feelings in the present. In a study with obese patients, 6 weeks of a

mindfulness-based eating intervention significantly decreased body weight compared to baseline, producing a mean weight-loss of 4kg at a 12 week follow-up (Dalen et al. 2010). Even very brief interventions might be beneficial to protect and maintain weight-loss. For example, patients completing a 6 month weight-loss program were either assigned to a one day mindfulness workshop, or placed on a waiting list (control group). At 3 months follow-up, a higher percentage of patients in the workshop group had lost weight, and fewer showed weight gain, compared to controls (Lillis et al. 2009). Hence, mindfulness might have some use in maintaining weight-loss, however, further longer-term follow-ups are needed to investigate this treatment.

1.3.4. Bariatric Surgery

Bariatric surgery, which includes gastric bypass, gastric banding and sleeve gastrectomy, is a particularly efficacious treatment for obesity, producing significant and reasonably fast reductions in BMI between 12 to 17kg/m^2 post-surgery (Chang et al, 2013). Gastric bypass involves compartmentalising the stomach into two pouches and rerouting a portion of the small intestine to the upper section of the stomach; Roux-en-Y Proximal is the most common gastric bypass procedure performed. The surgery results in enhanced feelings of fullness and satiety and is associated with reduced food intake (Elder and Wolfe, 2007).

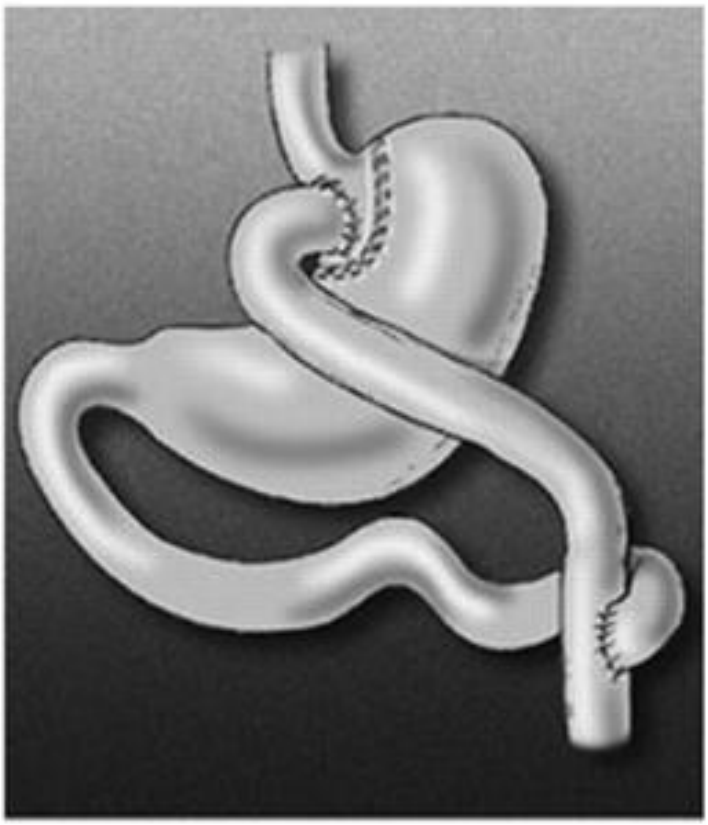

Figure 1.2 Roux-en-Y Gastric Bypass. The stomach is partitioned into two pouches; a small upper pouch and a lower larger pouch. A portion of the small intestine is then rerouted to the smaller upper pouch (image is from the American Society for Bariatric Surgery; www.asbs.org).

Gastric banding involves restricting the size of the stomach using a silicone band, thereby limiting the volume of food that can be ingested (Elder and Wolfe, 2007). Sleeve gastrectomy involves removal of the majority of the stomach, to permanently decrease stomach size, thereby restricting food intake (Elder and Wolfe, 2007).

While gastric restriction is a physical mechanism by which food intake and weight might be reduced, bariatric surgery has additional consequences. For instance, high serum leptin levels in obese patients are sharply reduced back to the normal range after biliopancreatic diversion (Adami et al. 1998), with similar results for gastric bypass surgery (Geloneze et al. 2003). In addition, ghrelin is also reduced by gastric bypass surgery, which has led to the hypothesis that ghrelin reductions might partly underlie decreases in food intake and body weight (Cummings et al. 2002; Geloneze et al. 2003). Hence, there are a range of hormonal effects that appear to contribute to the success of these methods.

A comparison of the effectiveness of surgical techniques showed that at 3 month follow-up, weight-loss was 35% for Roux-en-Y gastric bypass and 19% for gastric banding (Jan et al. 2005). However, at 36 months, weight-loss was 60% for Roux-en-Y gastric bypass, and 57% for gastric banding (Jan et al. 2005). In a comparison of sleeve gastrectomy versus Roux-en-Y, the latter showed a greater weight-loss after one year, however, after 2 years there was no significant difference between groups (Li et al. 2014). According to a meta-analysis, after 36 months treatment all bariatric surgeries produced at least 30kg weight-loss (Maggard et al. 2005). In a long-term follow-up of patients who had undergone gastric banding and gastric bypass surgery, mean weight-losses of 47-54% were maintained at 10-15 years (O'Brien et al. 2013). Compared to non-surgical treatments for obesity, a recent systematic review and meta-analysis of randomised controlled trials found that participants lost substantially more weight with bariatric surgery (mean difference = -26kg), and had higher remission rates of type 2 diabetes (Gloy et al. 2013). Hence, all forms of bariatric surgery provide similarly substantial levels of weight-loss but the effects are achieved more rapidly with gastric bypass.

Clearly, bariatric surgery can be extremely effective, however, it is not without issues. For certain populations, bariatric surgery is not effective in addressing the risk of mortality. Compared to patients who did not receive surgery, bariatric surgery did not decrease mortality in older severely obese individuals (Maciejewski et al. 2011). In addition, a recent systematic review and meta-analysis showed that 17% of patients experienced surgical complications and the mortality rate 30 days after bariatric surgery was 0.31% (Chang et al. 2014). Further, surgery does not directly address obesogenic eating behaviours such as the consumption of low-nutrient high-calorie foods. Therefore these maladaptive behaviours can persist post-surgery leading to nutritional deficiencies. (Xanthakos, 2009; Malinowski, 2006).

At present, bariatric surgery in the UK can either be accessed via the private health care system or is available without cost through the UK National Health Service (NHS) for patients meeting certain criteria. In the latter case, surgery is indicated when non-surgical interventions have proven ineffective with patients who have a BMI of 35-40 with comorbidities, or a BMI > 40 without comorbidities; it is also recommended as the first-line option for adults with a BMI > 50 (National Institute for Health and Care Excellence - NICE, 2006). Thus, individuals who do not meet these criteria but are obese have few options. Therefore, while bariatric surgery is effective at inducing weight-loss, the post-operative issues, and restrictions on eligibility, suggest that other forms of weight-loss interventions are required.

1.3.5. Pharmacotherapy

Weight-loss drugs have been used for decades, however, the vast majority of anti-obesity drugs have been withdrawn since they were launched. To understand the contribution of these

drugs to the treatment of obesity and the continued potential for anti-obesity drugs, the history and future of obesity pharmacotherapy will be reviewed.

1.3.5.1. History of Obesity Pharmacotherapy

Some of the earliest central nervous system (CNS) appetite suppressants were stimulants such as amphetamine, d-amphetamine and methamphetamine, which were used particularly in the 1950s and 1960s in the USA (Rasmussen, 2008). These drugs act via presynaptic release of dopamine and noradrenaline, producing sympathomimetic effects, appetite suppression and a decrease in food intake, cumulating in weight-loss (Foltin et al. 1990; Bray et al, 1992). The anorectic effect of amphetamine appears to be mediated by dopamine D_1 and D_2 receptors (Chen et al, 2001). Amphetamine produces 2.7kg weight-loss after 4 months treatment in obese patients (Haddock et al. 2002). However, due to their addictive properties and potential for abuse, the use of amphetamines is now controlled and restricted (Bray, 2001) and in the UK amphetamine is scheduled as a Class B illegal drug

Phentermine releases noradrenaline and dopamine, and was approved by the Food and Drug Administration (FDA) in 1959. Fenfluramine releases and inhibits the reuptake of serotonin, and was approved by the FDA in 1973. Dexfenfluramine (Redux) was approved by the FDA in 1996. Phen-fen, the combination of phentermine and fenfluramine, was not a marketed compound but the two drugs were often prescribed as a combination by physicians in the US. Fenfluramine and dexfenfluramine exert their anorectic effects through serotonergic mechanisms, particularly by an agonist action at the 5-HT_{2C} receptor (Vickers et al.1999; Vickers et al. 2001), which produces satiety enhancing effects, leading to reduced food intake in rats (for reviews see Dourish, 1995; Bickerdike et al. 1999). Phentermine stimulates

noradrenaline release, and to a lesser degree, dopamine release which is similar to the profile of amphetamine (Rothman et al. 2001). Phentermine has a particularly strong sympathomimetic effect, with significantly increased locomotor activity observed in rats (Rowley et al, 2000). The drug also has an effect on appetite, reducing hunger in healthy volunteers (Silverstone, 1972). Both fenfluramine and dexfenfluramine showed similar levels of weight-loss efficacy, in the region of 3.8kg after 8 months treatment (Haddock et al. 2002). Phentermine also showed comparable efficacy, producing a 3.6kg weight-loss at 6 months (Li et al. 2005). Fenfluramine and dexfenfluramine were withdrawn by the FDA in 1997 (Bowen et al. 1997) as their use is associated with cardiac valvulopathy caused by an agonist action at 5-HT_{2B} receptors expressed in cardiac tissue (Rothman et al. 2000).

Sibutramine received approval from the FDA as a new anti-obesity drug in the same year that fenfluramine and dexfenfluramine were withdrawn. Sibutramine is a serotonin-noradrenaline reuptake inhibitor that reduces food intake by accelerating satiety in humans (Halford et al. 2010). Agonist actions at α_1 and β_1 adrenoceptors and 5-HT_{2C} receptors probably mediate this satiety enhancing effect (Jackson et al. 1997; Higgs et al. 2011). Sibutramine-induced reductions in food intake are modest averaging approximately a 4.5kg after 12 months treatment (Barkeling et al. 2003; Li et al. 2005). In 2010, sibutramine was withdrawn from the market as it was discovered that the drug was associated with an increased risk of cardiovascular events, such as heart attack and stroke (Sayburn, 2010).

In 2006, the cannabinoid CB_1 receptor inverse agonist rimonabant was approved by the European Medicines Agency (EMA). Rimonabant reduces hunger and caloric intake in humans (Heshmati et al. 2001) and it has been claimed to have a stronger effect on the

consumption of palatable foods in rats (Arnone et al. 1997); though a recent study failed to replicate this finding (Buckley and Rasmussen, 2014). Rimonabant produced a 4.7kg weight-loss after 1 year (Christensen et al. 2007), which is similar in magnitude to the weight-loss caused by sibutramine. However, in January 2009 rimonabant was withdrawn from European markets due to adverse psychiatric effects including low mood, depression, and in some cases suicidal ideation (Butler and Korbonits, 2009). These side-effects prompted the FDA to issue a non-approvable letter for rimonabant for the US market.

The anti-obesity drug orlistat was approved as a prescription drug in 1999 and marketed as Xenical®. More recently, orlistat was approved by the FDA as an over the counter drug and is marketed as Alli™. Unlike most previous anti-obesity drugs that primarily targeted the CNS, orlistat is a pancreatic lipase inhibitor that prevents the breakdown and absorption of fat in the intestinal system (Davidson et al. 1999). This produces unpleasant gastro-intestinal side-effects, particularly for individuals consuming diets rich in fat (Finer, et al. 2000). In terms of efficacy, orlistat showed a weaker effect than sibutramine, reducing weight by approximately 2.9kg at 12 months (Li et al. 2005). Orlistat is presently the only licensed anti-obesity drug in the UK.

1.3.5.2. Future of Obesity Pharmacotherapy

Recently, the FDA has approved two new anti-obesity drug treatments; the selective 5-HT_{2C} receptor agonist lorcaserin (Belviq®) and a combination therapy of phentermine and topiramate (Qsymia®, formerly Qnexa). Lorcaserin is a full agonist at the 5-HT_{2C} receptor, and is 18 times more selective for the 5-HT_{2C} receptor than the 5-HT_{2A} receptor (Thomsen et al. 2008). The drug is also >100 fold more selective for the 5-HT_{2C} receptor than the 5-HT_{2B}

receptor, and all other 5-HT receptors (Thomsen et al. 2008). Lorcaserin reduces food intake in rats and this effect can be blocked by treatment with a selective 5-HT_{2C} receptor antagonist, but not a selective 5-HT_{2A} receptor antagonist, confirming the role of 5-HT_{2C} receptors in mediating the hypophagic effect (Thomsen et al. 2008). During an early clinical trial, 12 weeks treatment with lorcaserin 10mg twice daily produced weight-loss of 3.6kg in obese men and women (Smith et al. 2009). More recently, a meta-analysis of studies in which lorcaserin was administered for a year showed that lorcaserin produced a mean weight-loss of 3.2kg (Chan et al. 2013); an effect that is probably due to enhanced satiety and reduced food intake though this remains to be confirmed. Hence, 5-HT_{2C} receptor agonism remains a strategy to induce weight-loss pharmacologically. More broadly, there is likely to be continued interest in serotonergic drugs, as a recent study reported that serotonin transporter availability is associated with BMI (Hesse et al. 2014). Thus, the serotonergic system as a whole remains as an important target.

Qsymia appears to be more efficacious than lorcaserin and previous anti-obesity agents, inducing moderate weight-losses of 8.1-10.2 kg after a year of treatment (Gadde et al. 2011). While the behavioural mechanism and pharmacology underpinning these effects have been extensively investigated for phentermine, mediation of the effects of topiramate (initially developed and approved for the treatment of epilepsy) is less clear. Work with rodents suggests that topiramate increases energy expenditure and decreases food intake (Richard et al. 2000; Picard et al. 2000). In obese men topiramate decreases energy intake, but in the absence of any changes in appetite or satiety (Tremblay et al. 2007); hence, the mechanism responsible for reducing food consumption might be distinct from previous anti-obesity drugs. One possible explanation relates to the ability of topiramate to inhibit excitatory pathways through antagonism at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

(AMPA) receptor sites (Gibbs et al. 2000; Zullino et al. 2003). Indeed, as topiramate reduces neurotransmission of the excitatory neurotransmitter glutamate (Kaminski et al. 2004), and this has previously been linked to decreased food intake (Zheng et al.2002), it is possible that this is the primary pharmacological mechanism by which the drug affects energy intake. However, topiramate also potentiates gamma-aminobutyric acid (GABA) transmission, both acutely and chronically (Kuzniecky et al. 2002) and there is evidence that intracerebroventricular injections of GABA in rats decrease food intake (Olgiati et al. 1980), while in humans, the $GABA_B$ receptor agonist baclofen has been reported to produce a weight-loss (Arima and Oiso et al. 2010). Hence, the effect of topiramate on GABA neurotransmission might also play a significant role in its anti-obesity effects.

Unfortunately, compliance with Qsymia is affected by unpleasant side-effects of the drug, including: paresthesia; dry mouth; constipation; dysgeusia; and insomnia (Alison et al. 2011). Less frequent are: depression; irritability; alopecia; anxiety; disturbance in attention and hypothesia (Alison et al. 2011). Although licensed in the United States, the drug has recently been rejected by the EMA due to concerns about cardiovascular and CNS side-effects (2013). Hence, Qsymia is not yet approved for sale in the UK market.

Contrave, which is a combination of bupropion and naltrexone, has been submitted for FDA approval. Bupropion is a dopamine and noradrenaline reuptake inhibitor (Ferris et al. 1983) which enhances locomotor activity (Cooper et al, 1980), and decreases food intake in rats (Stairs and Dworkin, 2008) and induces weight-loss in humans (Anderson et al. 2002a). Naltrexone is an opioid receptor antagonist that reduces appetite and the rated palatability of foods by human volunteers (Speigel et al. 1987; Yeomans and Gray, 1997). Contrave

produces a more pronounced anorectic effect than either buproprion or naltrexone alone and appears to have fewer side-effects than buproprion when tested in rats (Wright and Rodgers, 2013). In humans, contrave produced modest reductions in body weight of 4.3 - 7.9kg at 56 weeks treatment (Apovian et al. 2013), suggesting it could be a useful anti-obesity agent.

Empatic is a combination of buproprion and zonisamide that is in development for the treatment of obesity. Zonisamide is another anti-epileptic drug with a similar pharmacological mechanism of action to topiramate (Ueda et al, 2003). Zonisamide also increases dopamine and serotonin levels (Okada et al, 1995; Okada et al, 1999) but the mechanism by which the drug decreases body weight is unclear at present. Zonisamide is associated with modest weight-losses of 7.3kg after a year (Gadde et al, 2012), whereas Empatic produces 8.1kg of weight-loss after only 12 weeks of treatment (Gadde, 2007).

There are also a large number of other compounds in earlier stages of preclinical and clinical development, targeting novel pathways (see Table 1.1. below). A number of neuropeptide receptors such as GLP-1, neuropeptide Y, and melanocortin are being investigated as potential targets, to treat obesity. Work on GLP-1 drugs has resulted in the GLP-1 agonist Exenatide (Byetta), and the GLP-1 analogue liraglutide (Victoza), which produces a sustained weight-loss of 7.8kg over 2 years, with minimal side-effects (Astrup et al. 2012). A neuropeptide Y receptor antagonist, Velneperit (George et al. 2014) and a melanocortin-4 receptor agonist RM-493 (Kievit et al. 2013) have also been reported to show early promise as weight-loss agents. In addition, a novel μ-opioid receptor antagonist GSK1521498 is currently in early stage clinical trials in humans (Ziaudeen et al. 2013).

Table 1.1 Promising novel anti-obesity drugs under development

Target	Drug	Mechanism of Action	Status
μ-opioid	GSK1521498	μ-opioid antagonist	Phase II
Melanocortin	RM-493	Selective MC4R agonist	Phase II
GLP-1	Liraglutide (Victoza)	GLP-1R Agonist	Phase III
	Exenatide (Byetta)	GLP-1R Agonist	Phase III
Neuropeptide Y	Velneperit	Y5 antagonist	Phase III

While the recent development and approval of new drugs to treat obesity is promising, the long term efficacy and safety of these compounds is still an area of ongoing research, and caution is clearly warranted (Li and Cheung 2009). For the development of future anti-obesity drugs, an emphasis on ensuring efficacy and safety is paramount. Thus, it is essential to develop models to help identify safe and effective drugs at an early stage of development, so that regulatory approval is not rapidly followed by drug withdrawal as was the case with dexfenfluramine in the US and rimonabant in Europe.

1.3.6. Summary

Obesity is a global issue, and there is an urgent need to develop new treatments that are effective in producing and maintaining weight-loss as safely as possible. Unfortunately, current treatments are not ideal. Dietary and exercise interventions show some weight-loss utility, however, maintenance of these effects is very poor. Psychological therapies such as CBT and mindfulness training might be useful in conjunction with lifestyle interventions but further work is needed to examine their utility in the long term. Bariatric surgery produces

rapid weight-loss and maintains weight-loss. However, there are significant post-operative problems including surgery mortality, post-surgical complications and malnutrition. The eligibility criteria used by the NHS in the UK for bariatric surgery also means that many obese individuals are unable to be treated until their BMI increases to the eligibility threshold. Therefore, a treatment that can be used as an alternative to bariatric surgery is urgently needed. The most logical treatment to fill this demand would be a highly efficacious and safe anti-obesity drug. While the history of obesity pharmacotherapy has been one of limited success, the recent approval of new drugs, and the development of novel agents is more encouraging. There is a clear need for novel approaches early in clinical development to ensure that future drugs are both efficacious in terms of weight-loss and safe in terms of psychiatric side-effects.

1.4. Experimental Medicine Model

1.4.1. Introduction

A barrier to successful development of new drug therapies for obesity is that the costs are high and the chance of a novel compound in Phase 1 trials reaching the market is very low, largely due to failure in late scale clinical trials or during the regulatory approval process (Kola and Landis 2004). Rimonabant is a prime example of a drug that was approved for use and then withdrawn shortly thereafter, resulting in a significant loss of time and money invested in its development (Butler and Korbonits 2009).

It is increasingly recognised by the pharmaceutical industry that the introduction of translational Experimental Medicine models at the interface between Phase 1 and Phase 2 clinical trials provides a more effective approach to assessing drug efficacy and safety before

large scale trials are undertaken (Dawson et al. 2011). Initial efficacy testing of novel compounds can also be conducted in healthy volunteers to assess safety issues that might pose risks for vulnerable patient populations (Dourish et al. 2008).

At present, the potential clinical efficacy of a compound to treat obesity is primarily assessed by weight-loss during Phase 3 Clinical Trials. Studies must demonstrate that either: (1) participants lose at least 5% of their baseline body weight after 1 year compared to placebo or; (2) at least 35% of participants lose at least 5% of their baseline body weight (FDA, 2007). Secondary efficacy measures that are also considered important include: blood pressure, pulse, lipoprotein lipids, fasting glucose and insulin, HbA1c in Type 2 diabetics and waist circumference. While these long term endpoints have obvious utility, they require lengthy periods of dosing, during costly and time-intensive studies. The ability to assess efficacy in shorter, acute studies could accelerate the development process. For example, sibutramine was marketed as an anti-obesity drug for over a decade, and induced weight-loss via a reduction in food intake (Barkeling et al. 2003). While the weight-loss effects take time to manifest, the effect of the drug on food intake are detectable after a relatively short period of dosing (7-14 days: Barkeling et al. 2003; Halford et al. 2010). As the sibutramine-induced reduction in food intake occurs acutely, and correlates with subsequent weight-loss (Barkeling et al. 2003), this suggests that measures of eating behaviour might provide an early/acute indicator of compound efficacy and assist in Go/No Go decisions during Phase 1 Clinical trials.

Studying the acute actions of anti-obesity agents can be useful in elucidating their behavioural mechanisms of action. This is important because there are many ways that drugs

can affect eating behaviour to induce weight-loss and some actions may be more effective than others. For example, a drug that enhances satiety may be preferable to a drug that has a general effect to decrease enjoyment of food and other rewarding stimuli. Hence, a model that can differentiate these various pathways would be extremely useful.

The requirements for ensuring psychiatric safety of compounds to treat obesity are less well defined by the regulatory authorities than those for weight-loss. The assessments that are used most commonly are validated neuropsychiatric questionnaires administered over lengthy weeks or months. The use of cognitive test batteries that measure emotional processing may offer an alternative approach to the assessment of psychiatric safety. These test batteries can be used in shorter term studies over days. For instance, the P1vital® Oxford Emotional Test Battery (ETB) is a computerised series of tasks that have been designed to assess cognition and emotional processing in human participants. In a study with rimonabant, the ETB detected negative effects of the drug on emotional processing after a single dose, in the absence of any change in standard questionnaire measures of mood, emotion, and psychiatric functioning (Horder, et al, 2009). Thus, the use of the ETB during phase 1 clinical trials might help to identify drugs with psychiatric safety concerns at an early stage of the clinical development process.

With regard to both efficacy and psychiatric safety, the use of fMRI to examine the effects of psychopharmacological agents is growing rapidly. Recent work with sibutramine showed that the drug reduced activity in the hypothalamus and amygdala (Fletcher et al, 2010, see below for further discussion). This suggests that fMRI might be a useful technique to detect changes in brain activity that are related to changes in eating behaviour and the long term outcome of

weight-loss. In addition, fMRI might be useful to assess psychiatric safety, as previous work has reported that depression is related to deficits in reward (anhedonia) circuitry (McCabe et al. 2009). Hence, if potential biomarkers of anhedonia, depression, or other psychiatric effects, are detectable using fMRI, it might be possible to identify such undesirable drug-induced effects at an early stage of testing.

1.4.2. Novel Techniques for Assessing Drug Efficacy and Psychiatric Safety

In developing an experimental medicine model to investigate anti-obesity drugs, there are a variety of potential methods and techniques that could be used to assess efficacy and psychiatric safety. Here, three specific methods will be reviewed: (1) Examining human eating behaviour using a Universal Eating Monitor (UEM) as an indicator of potential drug efficacy (2); Measuring emotional processing using the ETB as an indicator of psychiatric safety and; (3) Examining neural activity using fMRI as a potential indicator of both efficacy and psychiatric safety.

1.4.2.1. Measuring Eating Behaviour using a UEM

Total food intake is a key eating behaviour measure and could be a useful behavioural marker for anti-obesity drugs that aim to reduce food consumption. However, measurement of food intake alone does not provide any information on the behavioural mechanism by which anorectic drugs might exert their effects. Detailed microstructural examination of eating behaviour in rats was carried out in the 1970s and 1980s by Blundell and Colleagues (1981; 1985), who measured over various periods of time the amount of food consumed, latency to eat, duration of eating, number of eating bouts and local eating rate. This microstructural analysis of the individual components of a meal enables the detection of effects that are not

apparent at the macrostructural level. For instance, in a study in which rats were given five concentrations of sucrose, food deprivation did not affect the amount consumed of the two highest concentrations, however, it increased the duration of licking bouts, and decreased their number (Davis and Perez, 1993). Hence, changes can occur in the microstructure of ingestion patterns in the absence of changes in total food intake (Davis and Levine, 1977; Davis and Perez, 1993).

The use of microstructural measures has also proven particularly useful in understanding the effects of different types of drug on eating behaviour. For instance, the dopamine D_1 receptor agonist A-68930 and cocaine, reduce food intake due to selective reductions in the number of eating bouts (Cooper and van der Hoek, 1993; Al Nasar and Cooper, 1994). By comparison, amphetamine and fenfluramine reduce food consumption in rats via different microstructural actions: amphetamine increases inter-meal interval, whereas fenfluramine reduces eating rate (Blundell et al. 1976; Blundell, 1977; Blundell and Latham, 1978; Burton et al. 1981). More recently, it was reported that sibutramine selectively reduced consumption of glucose, by reducing the number of licking bouts (Higgs et al. 2011). Hence, different drugs can affect food intake via different microstructural actions. Therefore, it seems plausible that measurement of eating microstructure could be used to identify drugs that are effective anti-obesity agents based on positive changes to eating microstructure such as a reduced eating rate.

While the microstructural approach has been used in many rat studies to investigate the effects of anorexic drugs, there are fewer studies applying this approach to human volunteers. This is a key issue as effects in animal models do not always translate well to humans (see

Dourish et al. 2008; Dawson et al. 2011; Razafsha et al. 2013). A commonly used experimental setup is to present food buffets from which participants select a meal (Blundell et al. 1993; for a review of different experimental methods see Stubbs et al. 1998). These are particularly useful for examining the selection of different foods, however, they do not allow for microstructural analysis.

The UEM was developed by Kissileff and colleagues (1980) to record human eating behaviour during the course of a meal. The basic setup consists of a balance placed under a table, projecting through the surface. A placemat rests on top of the table, disguising the balance and providing a surface on which to place a plate of food. The balance is connected to a computer that records the food weight every few seconds as participants eat so that the microstructure of a meal can be analysed. This procedure was further adapted by Yeomans (1996) who configured a UEM system to incorporate visual analogue scale (VAS) ratings throughout the meal. In the Yeomans UEM system (the Sussex Ingestion pattern Monitor or SIPM), participants are presented with a dish of 200g pasta. After each 50g of food consumed, the computer interrupts participant to ask them to complete VAS ratings. After the participant has eaten 150g (75%) of the total 200g provided to them the participant is asked to stop eating so that the dish can be replaced with another serving. The participants can continue to eat for as long as they wish.

To date the UEM has been used to investigate various factors related to eating behaviour, including: the consumption of liquid versus solid meals (Kissileff et al. 1980); the effects of dietary restraint on eating (Westerterp-Plantenga et al. 1991); differences in eating patterns between bulimia nervosa patients and controls (Kissileff et al. 1996); the effects of food

palatability on eating (Yeomans, 1996); differences in eating patterns of lean and overweight individuals (Laessle et al. 2001); and as a test of the reliability of food intake measurement in the laboratory over time (Hubel et al. 2006). Yeomans (1996) also used the UEM to discover that when presented with highly palatable food, there is an initial stimulation of hunger and eating rate which occurs early within the meal, termed the 'appetizer effect'.

The UEM system has also been used to examine the actions of anorectic drugs. For instance, it has been reported that sibutramine treatment reduces the eating rate of obese women participants (Halford et al. 2010). Interestingly, 10 mg sibutramine significantly reduced intake after 12 minutes whereas 15mg sibutramine reduced intake after 4 minutes, suggesting that satiety was enhanced earlier in the meal at the higher dose. In addition, appetite VAS at the start and end of the meal were reduced by the 30mg and 15mg doses sibutramine respectively. In contrast, the opioid receptor antagonist naltrexone reduces food intake via a decrease in subjective ratings of food pleasantness and an abolition of the appetizer effect, suggesting a negative effect on food palatability (Yeomans and Gray, 1997). Hence, the UEM can distinguish drugs with different effects on appetite and satiety and has the potential to provide a behavioural marker of anti-obesity drug efficacy. However, further validation is needed to establish whether the UEM can be used successfully in acute single dose studies with healthy volunteers as most previous work has been carried out with clinical populations dosed for longer time periods.

1.4.2.2. Measuring Emotional Processing using the ETB

The ETB (see www.p1vital.com) comprises five validated cognitive tests that can be used to assess cognition and emotional processing (e.g. Murphy et al. 2008). The Facial Expression

Recognition Task (FERT) displays faces that participants must categorise into one of six emotional categories based on their expression: happiness; fear; anger; disgust; sadness; surprise; and neutral. Response accuracy, reaction time, response bias (bias towards one emotion over another) and target sensitivity (ease in detecting the target stimulus from other stimuli) can be calculated. The Faces Dot Probe Task (FDOT) involves the presentation of two faces, which are replaced by a pair of dots. On some trials, one of the faces has an emotional expression. Participants must report the orientation of the pair of dots (i.e. vertical versus horizontal). For this task, accuracy, reaction time and vigilance (sustained attention) can be calculated. The Emotional Categorisation Task (ECAT) displays positive and negative self-referent personality descriptors (e.g. "cheerful" versus "hostile", respectively) that participants must respond to, indicating whether they would like or dislike to be referred to as such. Accuracy and reaction times can be measured for this task. In the Emotional Recall Task (EREC) participants are asked to recall as many words as they can remember from the ECAT. This element is partly computerised: instructions given via computer, but words written down using pen and paper. The number of words recalled during this task can be calculated (both correct and incorrect). Finally, in the Emotional Recognition Memory Task (EMEM) words are re-presented from the ECAT, along with new distracter words, and participants are asked to report if they have previously seen the word. For this task, accuracy, reaction time, response bias and target sensitivity can be measured.

The EMEM and EREC provide measures of emotional memory while the FERT, FDOT and ECAT provide a measure of emotion-guided attention. Effects consistent with a negative effect on emotional responding would include reduced responses to positive stimuli, and or, enhanced responses to negative stimuli. For instance, compared to control subjects, depressed

individuals show: reduced accuracy in identifying happy facial expressions (FERT); increased reaction times to respond to positive emotional words (ECAT); and reduced recall of positive words (EREC) (Harmer et al. 2009).

Table 1.2 lists studies that have either used the ETB, or individual ETB tasks (e.g. FERT only). The ETB has been validated using a wide range of drugs, with different pharmacological mechanisms. Of the 28 studies listed, almost all showed drug-induced changes in either emotional processing or cognition, in the absence of any changes in standard measures of mood including VAS and standard psychiatric questionnaires, suggesting that the ETB is more sensitive to pharmacological manipulations than standard questionnaire measures. The ETB has also been used in several studies with clinical and clinical surrogate (e.g. dysphoric) populations, enabling the identification of behavioural markers of emotional processing. These include: individuals at risk of depression (Mannie et al. 2007; Chan et al. 2007; Le Masurier et al. 2007), anorexia nervosa (Jänsch et al. 2009), bipolar illness (Harmer et al. 2002b; Rock et al. 2010), panic disorder (Reinecke et al. 2013) and individuals exposed to postnatal depression (Douglas and Harmer, 2011). Hence, the ETB represents a single tool that can be used to identify markers of a number of relevant psychiatric disorders and test the potential of pharmacological agents to treat or exacerbate these conditions.

Table 1.2: Drugs studies using the ETB (or ETB Tasks)

Study	Drug(s) Tested
Harmer et al. 2013	GSK424887 (NK_1 antagonist + 5-HT reuptake inhibitor)
Mocking et al. 2013	Varenicline (Nicotinic acetylcholine receptor agonist)
Harmer et al. 2012	Negative Ion Treatment
Pringle et al. 2012	Memantine (NMDA antagonist)
Horder et al. 2012	Rimonabant (CB_1 inverse agonist)
Harmer et al. 2011	Agomelatine (Melatonin receptor agonist)
Browning et al. 2011	Citalopram (Selective serotonin reuptake inhibitor)
Pringle et al. 2011	Aprepitant (NK_1 antagonist)
Chandra et al. 2010	Aprepitant (NK_1 antagonist)
Di Simplicio et al. 2009	Oxytocin
Tranter et al. 2009	Citalopram and Reboxetine (Selective serotonin reuptake inhibitor and selective noradrenaline reuptake inhibitor)
Horder et al. 2009	Rimonabant (CB1 inverse agonist)
Arnone et al. 2009	Mirtazapine (Noradrenaline and serotonin antagonist)
Murphy et al. 2009	Citalopram and Reboxetine (Selective serotonin reuptake inhibitor and selective noradrenaline reuptake inhibitor)
Harmer et al. 2009	Reboxetine (Selective noradrenaline reuptake inhibitor)
Murphy et al. 2008	Diazepam (benzodiazepine agonist)
Harmer et al. 2008	Duloxetine (Serotonin-noradrenaline reuptake inhibitor)
Scrutton et al. 2007	Alpha-Lactalbumin (protein with high tryptophan content)
Murphy et al. 2006	Tryptophan supplementation
Harmer et al. 2006	Ondansetron ($5\text{-}HT_3$ receptor antagonist)
Hayward et al. 2005	Tryptophan depletion
Harmer et al. 2004	Citalopram and Reboxetine (Selective serotonin reuptake inhibitor and selective noradrenaline reuptake inhibitor)
Bhagwagar et al. 2004	Citalopram (Selective serotonin reuptake inhibitor)
Attenburrow et al. 2003	Tryptophan supplementation
Harmer et al. 2003b	Citalopram (Selective serotonin reuptake inhibitor)
Harmer et al. 2003a	Tryptophan depletion
Harmer et al. 2002a	Citalopram (Selective serotonin reuptake inhibitor)
Harmer et al. 2001	Propranolol (Non-selective beta adrenoceptor blocker)

An additional advantage of the ETB is that it can detect acute drug effects. For instance, several studies have reported that a single dose of the antidepressant citalopram in healthy volunteers produces changes in ETB task performance that are related to amelioration of clinical symptoms of depression (Harmer et al. 2002a; Harmer et al. 2003b; Bhagwagar et al. 2004). In addition, there is evidence that acute effects of antidepressant drugs on ETB tasks

in patients with depression are associated with clinical improvement after several weeks of treatment, suggesting predictive potential of acute results (Tranter et al. 2009). Furthermore, recent studies have demonstrated that administration of rimonabant, produces an increase in negative emotional bias after a single dose (Horder et al. 2009) and after 7 days treatment (Horder et al. 2012), in the absence of any changes in subjective mood measures. Thus, the ETB can detect negative psychiatric side-effects after a single dose, thereby allowing a rapid assessment of psychiatric safety of pharmacological interventions.

1.4.2.3. Measuring neural activity using fMRI

There are a number of techniques that can be used to investigate human brain activity. These include electroencephalography (EEG), magnetoencephalography (MEG), positron emission tomography (PET) and fMRI. Each method has advantages and disadvantages. For instance, EEG and MEG provide good temporal resolution but poor spatial resolution, whereas fMRI provides the opposite. In addition, all three of these techniques are non-invasive. PET is an invasive technique requiring injection of a radionuclide and has very poor temporal resolution. However, it has reasonable spatial resolution, and can be used to investigate metabolically active brain regions, or, can be used to investigate the engagement of specific neurotransmitter receptors (Huettel et al. 2009; Kumar et al. 2013).

For the investigation of anti-obesity drugs it is important that the neuroimaging technique provides a high spatial resolution of the neural circuitry affected by the drug. This is a key point because this level of detail is critical to interpret where the drug is affecting brain activity to produce changes in eating behaviour. It is also desirable to be able to assess specificity of responding to motivationally relevant stimuli (e.g. pictures or tastes of food)

versus control stimuli. Hence, currently the best available option to satisfy these requirements is fMRI as it provides good spatial resolution and the ability to examine neural responses to specific stimuli.

fMRI measures brain activity indirectly via blood oxygen levels (Ogawa et al.1990). When neurones become active at rest or during a task, local blood flow to those brain regions increases and oxygen-rich (oxygenated) blood displaces oxygen-depleted (deoxygenated) blood. Oxygen is carried by the haemoglobin molecule in red blood cells. Deoxygenated haemoglobin is more magnetic than oxygenated which is virtually nonmagnetic. This difference leads to an improved magnetic resonance signal, since the nonmagnetic blood causes less interference with the magnetic resonance signal. This improvement in resonance signal can be mapped using a magnetic resonance scanner to infer which brain regions are active at a particular time. By exposing participants to 'events' within the scanner such as pictures, tastes and smells, and recording the blood oxygen level dependent (BOLD) signal that encompasses this shift, it is possible to determine how exposure to specific stimuli affects brain activity (Huettel et al. 2009; Kumar et al. 2013).

Previous work has reported that fMRI can be used to detect changes in BOLD activity in a number of regions involved in the processing of appetitive stimuli, including: insula; orbitofrontal cortex; anterior cingulate cortex; ventral striatum; caudate; putamen; amygdala; hippocampus; and hypothalamus (Simmons et al. 2005; Kringelbach et al. 2003; Cornier et al. 2009; Killgore et al. 2003; Goldstone et al. 2009; Porubska et al. 2006; Stoeckel et al. 2008; Führer et al. 2008; LaBar. 2001). To date, neuroimaging has been used to: explore the effects of fasting on brain responses to food-related stimuli (St-Onge et al. 2005; Porubska et

al. 2006; Killgore et al.2003); compare the responses of lean and obese participants (Le et al. 2006); examine the differences in restrained and unrestrained eaters (Coletta et al. 2009) and; examine the neural substrate of sensory specific satiety (Kringelbach et al. 2003; Smeets et al. 2006).

The effects of gastrointestinal peptides and hormones on neural activity have also been investigated in recent years. For example, fMRI has been used to investigate the effects of leptin administration on the BOLD response. In leptin deficient patients, leptin replacement therapy reduced striatal activity to the sight of food images, indicating an effect of leptin on reward circuitry (Farooqi et al. 2007). The role of Peptide YY_{3-36} (PYY) has also been investigated in fMRI studies. In the presence of low concentrations of plasma PYY (that are associated with hunger) the hypothalamic BOLD signal predicts food intake. However, under high concentrations (that are associated with satiation) the OFC BOLD signal predicts food intake, potentially suggesting a switch from energy balance to reward mechanisms (Batterham et al. 2007). Ghrelin administration has also been reported to increase BOLD responses to the sight of food in the OFC, amygdala, insula, and striatum (Malik et al. 2008).

The effects of a number of anorectic drugs have also been examined in fMRI studies. Fletcher and colleagues (2010) examined the effect of sibutramine on fMRI BOLD responses to pictures of food. Sibutramine attenuated activity in the hypothalamus and this effect was correlated with both ad-libitum food intake and subsequent weight-loss. These data suggest that reduced hypothalamic activity is a potential biomarker of enhanced satiation and future weight-loss (Halford et al. 2010). Cambridge and colleagues (2013) reported that the novel μ-opioid receptor antagonist GSK1521498 decreased activation in the pallidum and putamen in

response to pictures of food. More recently, naltrexone was reported to reduce BOLD activity to rewarding chocolate stimuli in the dorsal anterior cingulate cortex and caudate (Murray et al. 2014). These data suggest that fMRI could prove useful in discriminating between anorectic drugs with different mechanisms of action. More broadly, the different patterns of activity observed for the opioid drugs and sibutramine suggests that fMRI data could be useful to investigate the neural mechanism of drug action, which for GSK1521498 and naltrexone implies an effect on reward processes, but for sibutramine suggests an effect on satiety processes.

Rimonabant decreases activation in the OFC and ventral striatum (key reward areas) in response to rewarding chocolate stimuli, and increases activation in the lateral OFC in response to aversive mouldy strawberry stimuli (Horder et al. 2010). This pattern of activity suggests a dampening of reward and a bias towards negative aversive stimuli, which may reflect the anhedonia and depression-like effects induced by the drug and is consistent with the results of studies conducted with rimonabant using the ETB (Horder et al. 2009; Horder et al. 2012). Thus, fMRI has the potential to assess both efficacy and safety in acute studies of potential compounds to treat obesity.

1.4.3. Summary

Developing new anti-obesity drugs requires the early assessment of both efficacy and psychiatric safety. The use of experimental medicine models during Phase 1 trials provides a potential approach to accomplish this objective. The use of behavioural methods to examine eating behaviour and emotional processing can enable the evaluation of efficacy and psychiatric effects in acute study designs with healthy volunteers. The supplementation of

this approach with fMRI can provide additional biomarkers that may reveal information about drug mechanism(s) of action.

1.5. Thesis Aims

The experimental work in this thesis is aimed at the development of an experimental medicine model to determine the potential efficacy and psychiatric safety of novel anti-obesity drugs. Thus, the thesis will aim to examine: (1) whether measures of eating behaviour provided by a UEM have utility as indicators of whether a drug will have a positive impact on eating behaviour, and elucidate a drug's neural and behavioural mechanism of action; (2) the utility of using measures of emotional processing obtained from the ETB to detect negative psychiatric side-effects; (3) whether using the fMRI BOLD signal would provide indications of drug mechanism and whether these measures might prove predictive of eating behaviour.

1.6. Thesis Hypotheses

The following overarching hypotheses were tested: (1) The UEM will be able to detect acute decreases in food consumption and eating rate of healthy volunteers after the administration of an anorectic agent (mCPP). (2) The ETB will be able to detect changes in mood in healthy volunteers (e.g. increased anxiety) after administration of a drug that was previously shown to induce such effects (mCPP). (3) Satiation and anorectic agents which are hypothesised to enhance satiety will decrease BOLD responses in reward circuitry in the brain of healthy volunteers.

CHAPTER 2: Study 1: Effects of the 5-HT_{2C} receptor agonist meta-chlorophenylpiperazine on appetite, food intake and emotional processing in healthy volunteers

2.1. Introduction

The aim of Study 1 was to assess the validity of using the Sussex Ingestion Pattern Monitor (SIPM) and the ETB to detect the potential efficacy and psychiatric safety of anti-obesity drugs. Previous studies examining drug effects with such a model are limited. A UEM has been used to examine the effects of the serotonin-noradrenaline reuptake inhibitor sibutramine on eating behaviour in humans: sibutramine was reported to reduce eating rate and enhance satiety (Barkeling et al., 2003; Halford et al., 2010). Using the ETB, Horder and colleagues (2009) tested the effect of the cannabinoid CB_1 receptor antagonist rimonabant on emotional processing in healthy volunteers. Rimonabant decreased positive emotional memory, which is a potential marker for the depressogenic effects (Butler and Korbonits, 2009).

To provide further validation of the UEM and ETB model it is necessary to test additional reference compounds. Here, the effects of the 5-HT_{2C} receptor agonist mCPP will be tested. mCPP is a metabolite of the anti-depressant trazodone, and while it is not used clinically for appetite suppression, it reduces food intake in rats (Kitchener and Dourish 1994; Kennett et al. 1994; Hewitt et al. 2002) and in humans (Walsh et al. 1994; Cowen et al. 1995; Sargent et al. 1997). mCPP can also increase anxiety in healthy volunteers and in patients with panic disorder (Charney et al. 1987; Kahn et al. 1990). Thus, mCPP provides both an opportunity for the UEM to detect an effect on eating behaviour and for the ETB to detect an effect on emotional responding.

In humans, mCPP shows the highest affinity for the 5-HT_{2C} receptor (compared to all other 5-HT receptors) with a binding affinity (K_i) of 3.4nM (Nelson et al. 1999) where it acts as an agonist (Thomas et al. 1996). mCPP is also an agonist at the 5-HT_{1B} receptor in rats, though it possesses a much lower affinity for 5-HT_{1B} receptors (Hoyer, 1988). In mice, the 5-HT_{1B} receptor agonist CP-94,253 suppresses food intake, but this effect is blunted or absent in 5-HT_{1B} knockout mice, or in animals pre-treated with a 5-HT_{1B} receptor antagonist (SB224289) (Lee et al. 2004). When dosed with a 5-HT_{1B} antagonist, mice show increased food intake (Lee et al. 2004). The effects of mCPP on food intake were compared to ORG 37684 (a relatively selective 5-HT_{2C} agonist) and CP-94,253 on food intake by Schreiber and De Vry (2002) who showed that mCPP produced the greatest reduction in food intake of the three compounds. The 5-HT_{2C} receptor antagonists metergoline and SB 242084 reversed the hypophagic effect of ORG 37684, but not the effect of mCPP or CP-94,253 (Schreiber and De Vry, 2002). Hence, at least part of mCPP's hypophagic effect is likely to be mediated by 5-HT_{1B} receptors in rodents. Humans, however, do not express the 5-HT_{1B} receptor in the brain. Therefore, it is likely that the hypophagic effects observed in humans are exerted via the 5-HT_{2C} receptor and this is worth bearing in mind when comparing the effects of mCPP in rodents and humans.

Participants were administered either a single dose of placebo, 15mg mCPP or 30mg mCPP, before consuming food from the UEM and then completing the ETB. Healthy volunteers were recruited and a between-subjects single-dose design was chosen. This is because in Experimental Medicine models it is more efficient to assess the potential efficacy of anti-obesity drugs at an early stage of development by investigating the acute effects of drug administration on behaviour. Furthermore, testing in healthy volunteers allows the assessment of safety issues that might pose risks for vulnerable patient populations (Dourish et al. 2008).

It was predicted that mCPP would decrease food intake and enhance microstructural measures of satiety as well as increase anxiety and negative mood as measured by the ETB.

2.2. Methods and Materials

Participants

48 healthy student volunteers (24 men and 24 four women) were recruited from the University of Birmingham; mean age 20.92 years (range 18-27), mean BMI 22.38 (range 18.93-26.30). The study was advertised via posters as an 'Appetite and Mood Study examining the effects of mCPP'. Participants received £80 compensation upon completion of the study. Informed consent was obtained and ethical approval was provided by the South Birmingham Research Ethics Committee (National Research Ethics Service). The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Participants were screened to exclude the following: under 18 or over 65 years old, BMI under 18.5 or over 27.5, English not first language as determined by the National Adult Reading Test (NART – Nelson 1982), taking psychotropic medication, past or current Axis 1 disorder as determined by the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID – Spitzer et al. 2004), pregnant or breast feeding, smoker, dyslexic, food allergies, vegan or vegetarian, diabetic, not using contraception (women), cognitive restraint score higher than 10 as measured by the Three Factor Eating Questionnaire (TFEQ - Stunkard and Messick 1985), and low rated pleasantness (<65) or low consumption of the test meal (<150g) on the food screening day. Women were not tested during their premenstrual week.

Design

The study used a parallel group, double-blind, placebo controlled design. A 30mg oral dose of mCPP was selected. Similar doses reduce appetite, and, in some cases, induce nausea and

anxiety (Sargent et al. 1997; Walsh et al. 1994; Cowen et al. 1995; Kahn et al. 1990). Therefore, a 15mg dose was also used, as this dose is not associated with nausea, but may still affect appetite (Smith et al. 1994a). The study had three conditions: placebo, 15mg mCPP, and 30mg mCPP. mCPP was supplied by the Guy's and St Thomas' NHS Foundation Trust Pharmacy Manufacturing Unit (GSTFT). Each group comprised 16 participants (8 men and 8 women). Both mCPP and placebo were prepared in identical capsules to maintain blinding.

Universal Eating Monitor (UEM)

Test meals were served on a UEM (the Sussex Ingestion Pattern Monitor - SIPM). This consisted of a balance (Sartorius Model CP4201; Sartorius Ltd, Epsom, UK; 0.1g accuracy) placed underneath, but protruding through, the surface of a table. A placemat on the table was used to hide the balance from view. The balance was connected to a laptop computer (refer to Figure 2.1). Using the procedure described by Yeomans (1996; 2000), plates containing 200g of pasta were placed on the mat and the amount of food eaten every 2 seconds was recorded covertly. The UEM software (SIPM Software version 2.0.8) was configured to interrupt participants each time they had eaten 50g of the meal and ask them to complete VAS ratings of hunger, fullness and pleasantness of the pasta. After 150g had been consumed, participants were interrupted and the plate was replaced with a fresh 200g plate of pasta. Participants were asked to eat in this manner until they felt 'comfortably full'. The test meal consisted of pasta shells in a tomato and herb sauce, both Sainsbury's U.K. own brand served at 55-60^{o}C (233 kcal per 200g serving).

For each participant, a text file was produced containing the computerised VAS results and a time course indicating the balance weight every 2 seconds. The following variables were generated: total amount eaten (g), eating rate (g/min) and average mouthful weight (g). Total

amount eaten was calculated via decreases in plate weight over the course of the meal (with the exception of plate removal). This figure was divided by the number of minutes spent eating to derive the eating rate. For average mouthful weight, the total amount eaten was divided by the number of times the balance recorded a weight change (i.e. a decrease in weight indicating that a forkful of food had been removed).

Figure 2.1 Left panel shows the UEM setup, featuring the laptop computer connected covertly to the balance underneath the red table mat with the dish for pasta placed on top. Right panel shows a standard 200g serving of the tomato-based pasta on the UEM setup.

P1vital® Oxford Emotional Test Battery (ETB)

The ETB is a computerised battery that comprises five validated cognitive tests see http://www.p1vital.com/Oxford%20Emotional%20Test%20Battery/index.html) which have been used in previous acute drug studies of emotional behaviour (e.g. Harmer et al. 2004; Murphy et al. 2008). The system produces an encrypted data file for each participant after they complete the test battery, which is then burned to a CD and sent to P1vital where it is decrypted and the data are processed (this procedure is used as security measure). The data processing generates all of the outcome measures, including: accuracy, reaction time, response bias, target sensitivity and vigilance score.

Accuracy is a measure of correct responses and is expressed as the percentage of correct responses of the total responses made. Reaction time is a measure of how long participants take to respond to a stimulus by pressing a button on the ETB button box and is expressed in milliseconds. Response bias measures the tendency to respond more or less to one stimulus than another (thus a bias can be positive or negative) and is calculated by taking into account the number of false alarms (when participants incorrectly respond that a stimulus is present) and misses (when participants incorrectly respond that a stimulus is not present). Target sensitivity measures the ease with which participants are able to detect a target stimulus against background distracter stimuli, by using accuracy and false alarms. Finally the vigilance score is a measure of sustained attention for a given stimulus, and is calculated by subtracting the reaction times from congruent trials (trials where the probe appears in the same location as the stimulus) from incongruent trials (trials where the probe appears in a different location from the stimulus).

Facial expression recognition task (FERT): Faces with one of six emotional expressions (happiness, fear, anger, disgust, sadness and surprise) or a neutral expression were displayed. The pictures (from Ekman and Friesen 1976) were morphed from neutral to 100% expressions (Young et al. 1997) in 10% stages, producing 10 intensities for each emotion. Each intensity was presented four times for each emotion, along with 10 presentations of neutral expressions, yielding 250 stimuli. These were presented for 500ms, followed by a blank screen. Participants classified each expression as quickly and accurately as possible, using the button box provided. Accuracy, response bias (bias towards one emotion over another) and target sensitivity (ease in detecting the target stimulus from other stimuli) were calculated.

Faces dot probe task (FDOT): Pairs of photographs (from Matsumoto and Ekman 1988) were presented comprising either: one emotional (either happy or fearful) and one neutral facial expression, or two neutral expressions. Faces appeared above or below a central fixation point. For unmasked trials the pair was displayed for 100ms, whereas for masked trials, the pair was displayed for 16ms, and then replaced with a mask for 84ms; for both trials, the images were then replaced with a probe, located in the position of one of the faces (or masks). The probe consisted of a pair of dots, in either a vertical (:) or horizontal (‥) orientation. Participants indicated the orientation as quickly and accurately as possible using the corresponding buttons on the button box. There were 192 trials: 32 happy + neutral; 32 fear + neutral; 32 neutral + neutral for both masked and unmasked trials. Vigilance (sustained attention) scores were calculated.

Emotional categorisation task (ECAT): Sixty positive and negative self-referent personality descriptors (e.g. "cheerful" versus "hostile") (Anderson 1968), were displayed for 500ms. Words were matched for meaningfulness, length and frequency of occurrence. Participants indicated using the button box whether they would like or dislike to be described as such as quickly and accurately as possible. Accuracy and reaction times were measured.

Emotional recall task (EREC): participants were asked to recall as many words as they could remember from the ECAT, within a four minute period. This task was partly computerised; instructions given via computer, but words written down using pen and paper. The number of correct words recalled and their valence was measured.

Emotional recognition memory task (EMEM): Participants were presented with a series of words on a computer screen, containing the 60 personality descriptors from the ECAT, along

with 60 matching novel distracter words. Participants were instructed to use the button box, pressing 'yes' or 'no' to indicate whether the word had been presented during the ECAT. Accuracy, response bias and target sensitivity were calculated.

Biological samples

Salivary cortisol: Saliva was collected using salivettes (SARSTEDT). Participants abstained from drinking water for 30 minutes prior to giving the sample and then chewed on a synthetic cotton wool swab for 60 seconds. The sample was then taken to be centrifuged for 2 minutes at 1000g and then frozen at -80ºC until analysed. Salivary cortisol was measured by liquid chromatography-mass spectrometry (LC-MS/MS) by the Clinical Laboratory Services, University Hospitals Birmingham NHS Foundation Trust. A Waters Aquity UPLC system was used for chromatography and was connected to a Waters Premier XE tandem mass spectrometer with an electrospray ion source. Intra-assay coefficients of variation (CVs) were <5.9% and inter-assay CVs <9.8% between 3 and 47 nmol/L. The lower limit of quantification, defined as the lowest concentration at which the CV is <20%, was 1.6 nmol/L.

Procedure

The experimental procedure is summarised in Figure 2.2.

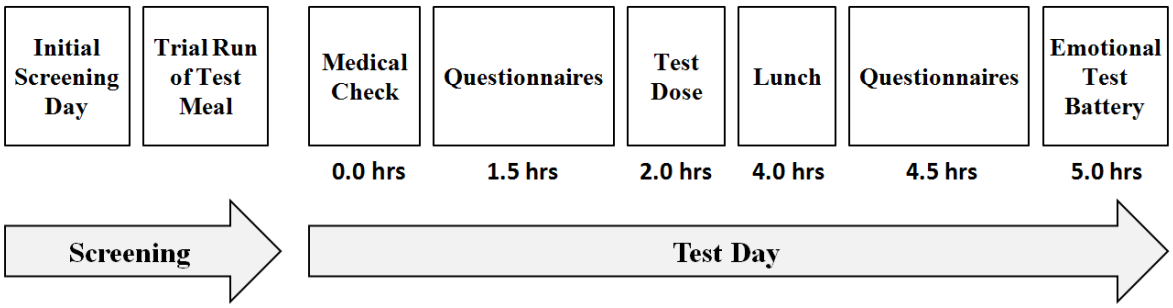

Figure 2.2 Flow diagram for screening process followed by an overview of key events and timings for test days in hours (hrs).

Screening Days: Participants underwent an initial screening at the School of Psychology, University of Birmingham for exclusions and completed the Eysenck Personality Questionnaire (EPQ - Eysenck and Eysenck 1975) to determine baseline personality traits. They returned approximately one week later for a trial run with the test meal served; those who liked and ate a sufficient amount of the pasta proceeded to the test day.

Test Day: Participants arrived at the Welcome Trust Clinical Research Facility (WTCRF, University Hospitals Birmingham NHS Foundation Trust - Queen Elizabeth Hospital) at 8.00, 9.00 or 10.00am. It was ensured that participants had eaten their normal breakfast two hours earlier by asking them to complete a breakfast questionnaire detailing their food intake that morning. Participants then completed a physical health check with a physician. This examination included blood pressure measurement and an electrocardiogram (ECG). They were then breathalysed and women completed a pregnancy test. All participants passed these checks. Participants also completed baseline VAS ratings of mood and appetite that comprised rated: 'alertness', 'disgust', 'drowsiness', 'light-headed', 'anxiety', 'happiness', 'nausea', 'sadness', 'withdrawn', 'faint', 'hungry', 'full', 'desire to eat' and 'thirst'. VAS scales comprised 100mm horizontal lines, anchored to the left and right with 0 and 10 respectively. Participants were asked to place a vertical line on the scale to indicate how they felt at the moment, with 10 being the most they could ever imagine and 0 being completely absent. 1.5 hours after the start of the session, participants completed a pre-dose Beck Depression Inventory (BDI – Beck et al. 1961), Befindlichskeit scale of mood and energy (BFS - von Zerssen et al. 1974), and Positive and Negative Affective Schedule (PANAS – Watson et al. 1988), as measures of subjective mood and energy. They also completed a State Trait Anxiety Inventory (STAI – Spielberger, 1983) as an index of their anxiety, and another set of VAS as described above. After 2.5 hours, further VAS were completed and a saliva

sample was taken, after which participants were administered the test dose; either placebo, 15mg mCPP or 30mg mCPP. VAS were completed every 30 minutes. 4.5 hours after the start of the session, participants were given ad-libitum access to the test meal on the UEM, and completed the computerised VAS during the meal. Following the meal, participants completed: BDI, BFS, PANAS, STAI and VAS. A saliva sample was taken an hour after lunch and participants then completed the ETB, which took approximately 90 minutes. A final set of VAS and a saliva sample were taken at the end of the session, along with a single blood sample. Participants were fully debriefed and questioned to determine if they were aware of the hidden balance or the aims of the study.

Data Analysis

General: One participant did not complete testing and so their data were removed from all analyses (woman 30mg mCPP group). For statistical analyses, effects and interactions with condition were determined by analysis of variance (ANOVA) and followed up with planned comparisons. Interactions with condition were also analysed if they approached statistical significance. Dunnett's correction was used for all t-tests, and violations of sphericity were addressed using the Greenhouse-Geisser correction.

VAS: To establish a factor structure for the VAS, a principal components analysis (PCA) was run with varimax rotation. All of the visual analogue scales data (i.e. all data points from all time points across the testing session – both between and within-subjects data) were entered into the PCA. Analysis of the 14 items provided 5 factors with eigenvalues > 1, accounting for 70.81% of the variance. Items that loaded > 0.5 onto a factor were included, resulting in four factors of 3 or more items: appetite (hunger, fullness and desire to eat), physical effects (lightheaded, nausea and faint), negative effects (disgust, anxiety, sadness, withdrawn and

thirst) and arousal (alertness, drowsiness and happiness). Scores for each of the factors were calculated by summing the scores for all items in that factor, and then dividing by the number of items. Items with a negative scale, were inverted to match the other items.

UEM: For the UEM, microstructural data for 12 participants were lost due to technical issues with the balance and so the data for 35 participants were analysed. For total intake and correlational analysis, an estimate of intake was used. For instance, where intake data were missing for a plate, it was assumed that if the plate was finished, the individual had eaten approximately 150g.

2.3. Results

Participant Characteristics

The groups did not differ in age, BMI or EPQ scores (all $p > 0.05$). There was a main effect of gender for the NART, with men scoring higher scores than women (F (1 41) = 5.12; $p < 0.05$) and for the TFEQ factor cognitive restraint, with women showing higher restraint than men (F (1 41) = 4.19; $p < 0.05$), but there were no other main effects or interactions at baseline (Table 2.1).

Table 2.1 Participant characteristics, baseline means, and test meal intake (standard error of the mean)

	Men			Women		
	Placebo	15mg	30mg	Placebo	15mg	30mg
Gender	8	8	8	8	8	7
UEM Data	5	5	5	8	7	5
Age	20.8 (0.4)	21.9 (0.8)	20.4 (0.5)	22.4 (1.0)	20.4 (0.5)	19.9 (0.7)
BMI	23.8 (0.7)	22.1 (0.7)	22.8 (0.8)	21.5 (0.7)	22.0 (0.8)	22.4 (0.9)
NART	114.9 (1.0)	114.6 (1.7)	112.6 (1.6)	112.9 (1.0)	111.9 (1.6)	109.7 (0.9)
TFEQ CR	4.3 (1.4)	3.6 (1.0)	3.1 (1.0)	6.9 (0.9)	5.5 (1.1)	4.1 (1.2)
EPQ N	4.0 (0.8)	4.0 (1.0)	4.0 (1.0)	7.9 (1.1)	3.3 (1.3)	4.1 (0.6)
Test Meal	649.3 (90.1)	778.2 (111.6)	663.8 (107.7)	510.1 (60.2)	442.8 (31.1)	409.2 (66.9)

BMI - Body Mass Index; NART - National Adult Reading Test; TFEQ - Three Factor Eating Questionnaire (CR - Cognitive Restraint); EPQ - Eysenck Personality Questionnaire (N - Neuroticism)

Salivary Cortisol

Salivary cortisol was analysed by gender, condition, and time (baseline measure: *t*0; time after dosing: *t*60, t120, t180, t300). There was a main effect of time (F (4 164) = 7.07; $p < 0.001$), condition (F (2 41) = 12.32; $p < 0.001$), an interaction between condition and time (F (8 164) = 2.49; $p < 0.05$), and an interaction between time and gender that was not analysed further (F (4 164) = 3.42; $p < 0.05$). Post hoc analysis showed there were main effects of condition for t60 (F (2 44) = 7.41; $p < 0.01$), t120 (F (2 44) = 8.37; $p < 0.01$) and t300 (F (2 44) = 6.16; $p < 0.01$). Cortisol was significantly higher in the 15mg mCPP condition compared to placebo at *t*60 (t (30) -2.43; $p < 0.05$); while cortisol was higher in the 30mg mCPP condition compared to placebo for *t*60 (t (29) -4.18; $p < 0.01$), *t*120 (t (29) -3.86; $p < 0.01$) and *t*300 (t (29) -3.36; $p < 0.01$) (Figure 2.3). There was no interaction between gender and condition (F (2 41) = 0.32; p > 0.05), or gender, condition and time (F (8 164) = 3.29; p > 0.05), indicating that there was no differential cortisol response for men versus women to mCPP.

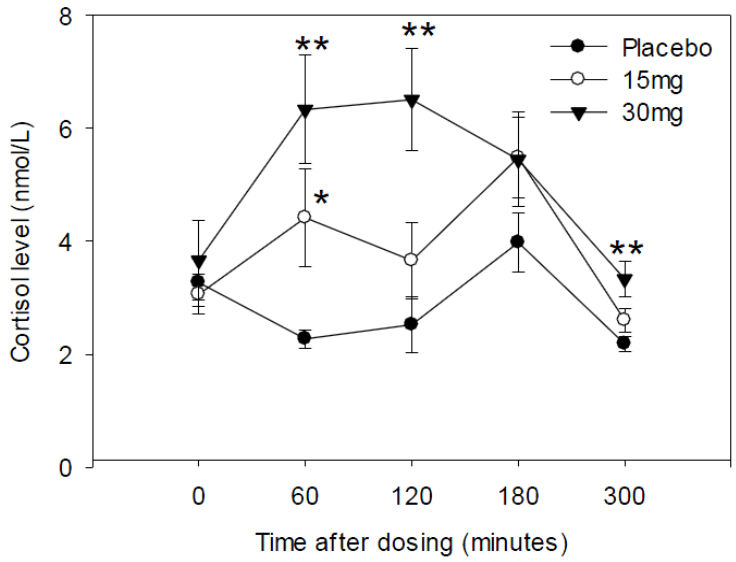

Figure 2.3 Salivary cortisol levels over time for placebo, 15mg mCPP, and 30mg mCPP. The 15mg group showed a significant increase in salivary cortisol compared to the placebo group at *t*60, and the 30mg group showed an increase at *t*60, *t*120 and t300. Error bars represent standard error of the mean. *$p < 0.05$; **$p < 0.01$

Subjective State Questionnaires

Data were analysed by gender, condition, and time (pre versus post-dosing). There were no main effects or interactions for the BDI, STAI Trait and State, or PANAS negative scores (all $p > 0.05$). There was, however, a main effect of time for PANAS Positive scores, with a small decrease in positive affect over the day (from 30.43 to 28.11; F (1 41) = 9.64; $p <$ 0.01). For the BFS, there a significant interaction between time and condition (F (1 42) = 4.363; $p < 0.05$), whereby the placebo group BFS score decreased over time (t (15) 2.18; $p =$ 0.05).

Visual Analogue Scales (VAS)

Pre-dosing VAS scores were averaged for a pre-dose baseline. Each factor (negative effects, arousal, appetite, and physical effects) was analysed separately, by condition and gender,

showing no main effects or interactions (all $p > 0.05$). Post-dosing factors were then analysed by gender, condition and time (post-dosing in minutes: *t*30, *t*60, *t*90, *t*120, *t*150, *t*210).

Negative effects and Arousal: There were no main effects or interactions for negative effects (all $p > 0.05$). For arousal, there were no main effects (all $p > 0.05$), but a significant two way interaction between time and condition (F (5 107) = 2.37; $p < 0.05$); follow up tests were not statistically significant (all $p > 0.05$), though there was a near significant effect of condition for *t*210 (F (2 44) = 2.67; $p = 0.08$), with mean arousal scores of; 64.69 for placebo, 75.79 for 15mg mCPP and 75.22 for 30mg mCPP (see Figure 2.4).

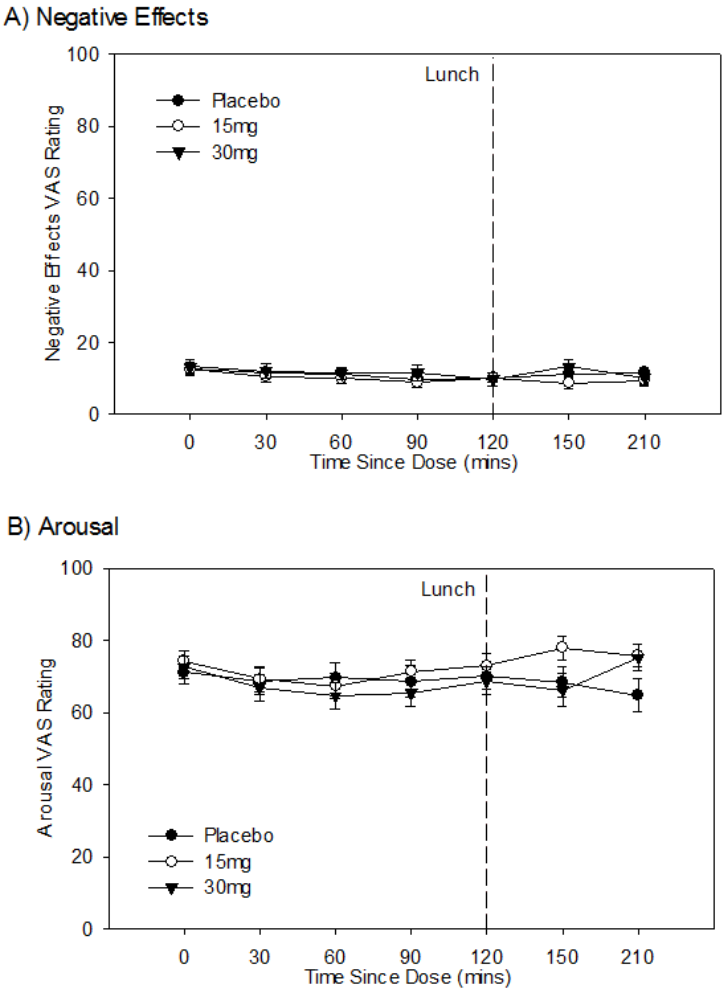

Figure 2.4 Effect of mCPP on negative effects and arousal VAS ratings. For negative effects (Panel A) there were no main effects or interaction. For Arousal (Panel B), there were no main effects but a significant interaction between time and condition – post hoc tests did not show any significant differences in condition at any time points, though there was a trend for an effect at *t*210, with higher mean scores in the drug conditions compared to placebo. Error bars represent standard error of the mean.

Physical Effects: There was a main effect of time (F (3 117) = 160.94; $p < 0.01$) and condition (F (2 41) = 5.97; $p < 0.01$ see Figure 2.5), and a significant two way interaction (F (6 117) = 3.25; $p < 0.01$). Main effects of condition were observed for: *t*60 (F (2 44) = 6.62; $p < 0.01$), *t*90 (F (2 44) = 7.83; $p < 0.01$) and *t*120 (F (2 44) = 4.69; $p < 0.05$), with physical effects rated significantly higher in the 30mg mCPP group than placebo (all $p < 0.05$ – Figure 2.5).

Appetite: There was a main effect of time (F (3 115) = 237.00; $p < 0.001$) and condition (F (2 41) = 3.78; $p < 0.05$ see Figure 2.5) but no significant interaction. For the effect of time, all comparisons were significantly different (all $p < 0.05$), and for condition, appetite was significantly lower in the 30mg mCPP group than placebo (t (29) 2.30; $p < 0.05$ - Figure 2.5).

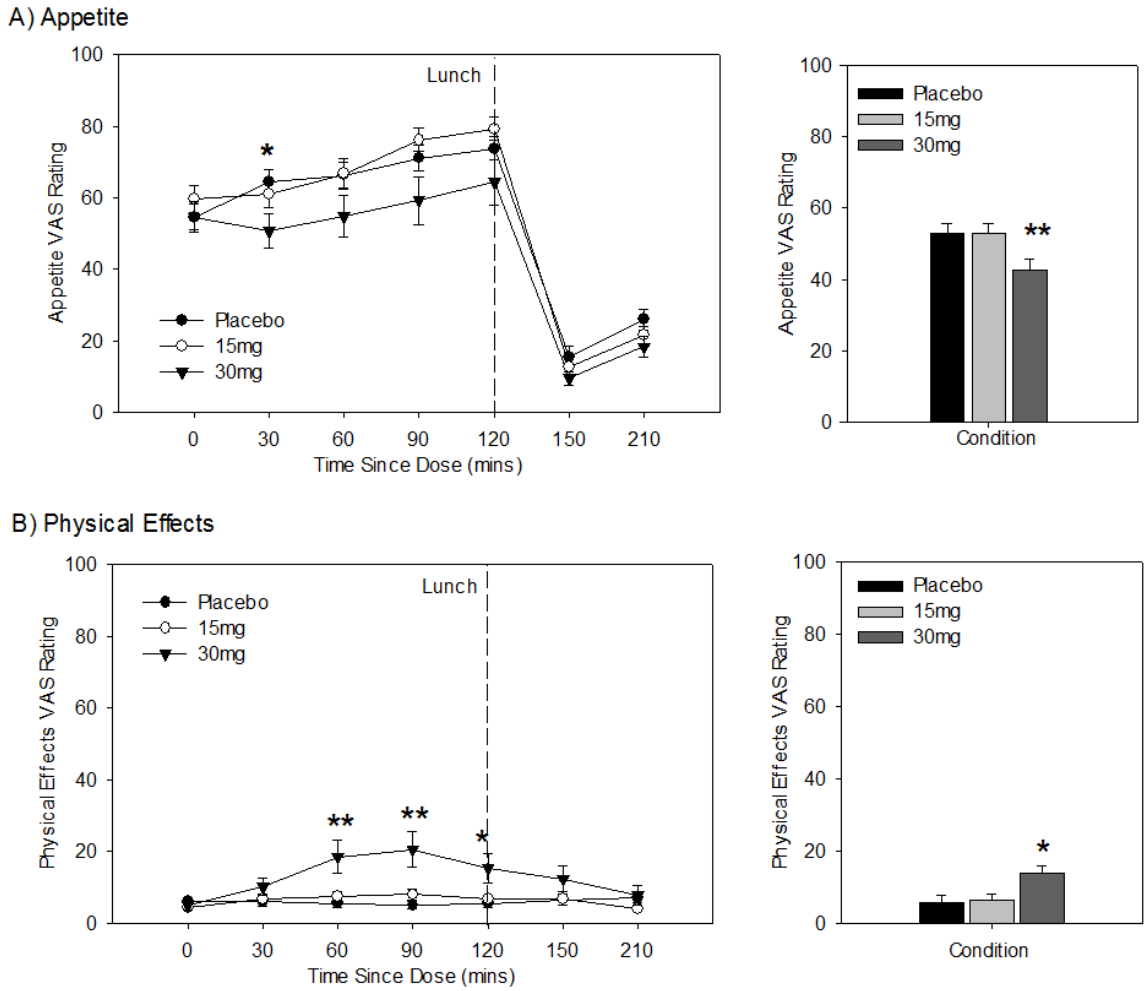

Figure 2.5 Effect of mCPP on appetite and physical effects VAS ratings. For appetite (Panel A) there was a main effect of condition (**histogram on right panel)** where appetite was significantly lower in the 30mg group compared to the placebo group**,** but no significant interaction (**graph on left panel** – post hoc t-tests showing a difference between placebo and 30mg at *t*30 only). Physical effects VAS scores (Panel B) showed a main effect of condition (**histogram on right panel**) and an interaction between condition and time (**graph on left panel),** where physical effects were significantly higher for the 30mg group at *t*60, *t*190 and *t*120, compared to placebo. Error bars represent standard error of the mean. *$p < 0.05$; **$p < 0.01$

Meal measures

Test meal intake: There was a main effect of gender only, with men eating more than women (F (1 29) = 11.32; $p < 0.01$). There was no effect of condition, nor any interaction (Table 2.1).

Meal Intake quartiles: Amount eaten was analysed by quartile, gender, and condition, showing a main effect of quartile (F (3 87) = 8.18; $p < 0.01$), gender (F (1 29) = 11.32; $p < 0.01$) and a two way interaction between quartile and condition (F (6 87) = 2.56; $p < 0.05$) although follow up tests were not significant (see Table 2.2 for means). Men ate more than women (174.09 grams versus 106.57 grams). Men had a faster eating rate than women (105.72g/min versus 67.23g/min; F (1 29) = 13.51; $p < 0.01$) and ate larger mouthfuls than women (12.06 grams versus 7.76 grams; F (1 29) = 6.56; $p < 0.05$).

Table 2.2 UEM measures split by condition and quartile (standard error of the mean)

	Placebo				15mg				30mg			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Amount Eaten	117.3 (19.2)	120.5 (18.9)	120.6 (20.3)	125.9 (18.8)	137.1 (19.9)	135.9 (19.7)	141.2 (21.1)	146.4 (19.6)	140.4 (21.8)	158.1 (21.6)	140.9 (23.1)	168.0 (21.5)
Eating Rate (g/min)	84.7 (8.7)	78.8 (12.9)	76.5 (9.8)	79.0 (12.7)	74.8 (8.9)	84.1 (13.3)	81.6 (10.1)	61.8 (13.1)	98.2 (9.6)	124.7 (14.3)	95.0 (10.9)	98.4 (14.1)
Mouthful Weight (g)	10.1 (1.3)	7.8 (1.1)	8.6 (0.8)	8.6 (4.9)	7.2 (1.3)	8.4 (1.1)	9.9 (0.8)	17.6 (5.1)	8.9 (1.4)	11.4 (1.2)	9.7 (0.9)	10.8 (5.5)

Computerised VAS: Hunger decreased across quartiles (F (2 50) = 150.46; $p < 0.001$), and men were more hungry than women (47mm versus 38mm, respectively; (F (1 29) = 3.81; $p = 0.06$) (all $p < 0.001$). Similarly, fullness increased across quartiles (F (2 47) = 176.78; $p < 0.001$), and men were less full than women; (59mm versus 51mm, respectively; (F (1 29) =

4.46; $p < 0.05$)). For pleasantness, there was a main effect of quartile only (F (2 45) = 31.65; $p < 0.001$), with rated pleasantness decreasing across quartiles (all $p < 0.05$).

Satiation Quotient

The satiety quotient (Green et al. 1997 (pre-meal hunger – post meal hunger) / calories consumed) provides a measure of how satiating a given amount of calories is at various time points after an eating episode has finished. Adapting this, a satiation quotient (SQ) per quartile was calculated; a measure reflecting the satiating capacity of a food as it is eaten (quartile initial hunger – quartile ending hunger rating) / calories consumed during quartile). There was a main effect of quartile (F (3 87) = 11.62; $p < 0.001$) and gender (F (1 29) = 7.11; $p < 0.05$), a two way interaction between gender and condition (F (2 29) = 8.61; $p < 0.01$), and a three way interaction between quartile, gender and condition (F (6 87) = 2.50; $p < 0.05$). For men, there was an effect of quartile (F (3 36) = 6.87; $p < 0.01$) and condition (F (2 12) = 4.30; $p < 0.05$). SQs were significantly lower in the 30mg mCPP group compared to placebo (t (8) = 2.72; $p < 0.05$) and there was a significant increase in SQ from quartile 2 to 3 (t (14) -2.86; $p < 0.05$). For women there was a main effect of quartile (F (3 51) = 6.53; $p < 0.01$), condition (F (2 17) = 4.84; $p < 0.05$) and an interaction between quartile and condition (F (6 51) = 2.58; $p < 0.05$). Breaking down the interaction by quartile; for quartile 1, SQ was significantly higher for 30mg mCPP than placebo (t (11) = -2.44; $p < 0.05$); and for quartile 2, SQ was significantly higher for both 15mg mCPP and 30mg mCPP, compared to placebo (t (13) = -3.07; $p < 0.01$; t (11) = -2.69; $p < 0.05$) (Table 2.3).

Table 2.3 Mean satiation quotients by quartile and condition for women participants (standard error of the mean)

	Condition		
Quartile	Placebo	15mg	30mg
1	0.04 (0.04)	0.04 (0.02)	0.20 (0.05)*
2	0.10 (0.02)	0.21 (0.03)**	0.21 (0.04)*
3	0.18 (0.05)	0.21 (0.05)	0.46 (0.16)
4	0.17 (0.04)	0.20 (0.06)	0.09 (0.02)

Compared to placebo: * $p < 0.05$; ** $p < 0.01$

Correlations

To investigate the relationship between appetite, physical symptoms (both measured prior to lunch) and total food intake, correlations were performed on the three measures. A positive correlation was identified between appetite and intake (r (47) = 0.32, $p < 0.05$), but there was no association between physical symptoms and intake, or between physical symptoms and appetite (r (47) = -0.05, $p > 0.05$; (r (47) = -0.15, $p < 0.05$).

Emotional Test Battery (ETB) Data

Facial expression recognition task (FERT) and Faces dot probe task (FDOT): There were main effects of emotion (F (3 140) = 35.46; $p < 0.001$; F (6 246) = 130.28; $p < 0.001$) but no other significant effects or interactions for target sensitivity and response bias (all $p > 0.05$). There were no significant main effects or interactions for vigilance scores (all $p > 0.05$).

Emotional categorisation task (ECAT): Accuracy data showed a main effect of gender only, with women showing greater accuracy than men (96.70% versus 89.34%, respectively; F (1 40) 4.36; $p < 0.05$). Reaction time showed a main effect of valence only, with longer reaction times to negative versus positive stimuli (887.36ms versus 825.36ms, respectively; F (1 40) 19.47; $p < 0.001$).

Emotional recall task (EREC): More positive than negative words were recalled (5.8 versus 3.8 words; F (1 41) = 38.97; $p < 0.001$). There was also a main effect of condition (F (2 41) = 4.77; $p < 0.05$) with more words correctly recalled in the 15mg mCPP and the 30mg mCPP groups than placebo (5.1 and 5.6 versus 3.6, respectively) (both $p < 0.05$ see Figure 2.6).

Emotional recognition memory task (EMEM): Target sensitivity showed a main effect of condition only (F (1 41) = 5.87; $p < 0.01$), whereby target sensitivity was significantly higher in the 30mg mCPP group than placebo (t (23) -4.31; $p < 0.001$ see Figure 2.6).

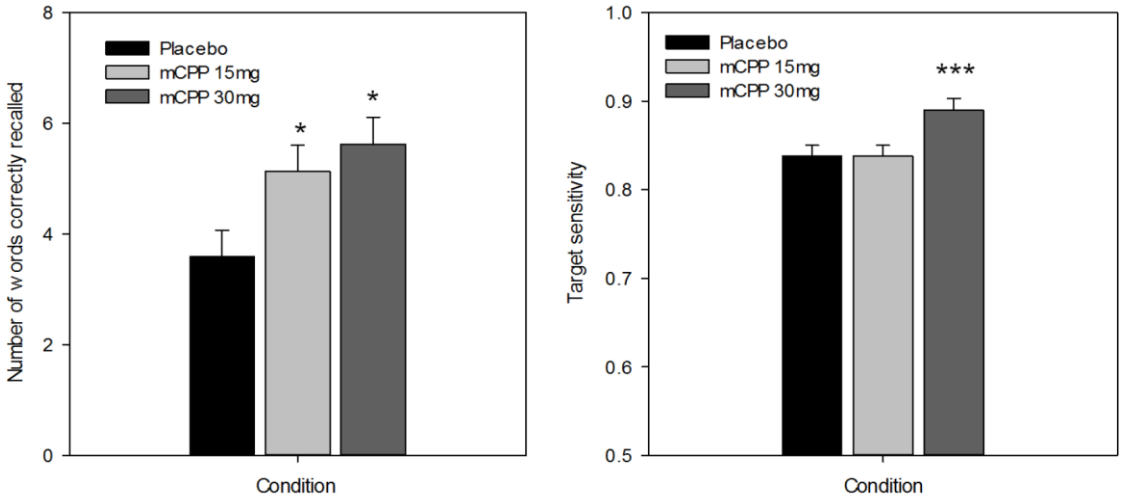

Figure 2.6 Mean number of words correctly recalled (**left panel**) and mean target sensitivity (**right panel**) for placebo, 15mg mCPP and 30mg mCPP groups in the emotional recall task. Word recall was enhanced in both the 15mg and 30mg groups compared to placebo and target sensitivity was enhanced in the 30mg group compared to placebo. Error bars represent standard error of the mean. *$p < 0.05$; ***$p < 0.001$

2.4. Discussion

The 5-HT_{2C} receptor agonist mCPP at doses of 15mg and 30mg decreased appetite ratings of both healthy men and women, and enhanced within-meal satiation quotients (SQs) of women participants in a dose-related manner but had no significant effect on food intake. mCPP also dose-dependently increased ratings of negative physical effects, in particular nausea, but had no effects on ratings of negative mood or cognitive measures of anxiety and depression as

assessed by the ETB, for men and women participants. However, mCPP enhanced memory for emotional words independent of their valence.

As expected, mCPP dose-dependently increased salivary cortisol, confirming activation of 5-HT_{2C} receptors. Peak effects for both doses occurred 60-180 minutes post dosing, which is consistent with previous reports (Kahn et al. 1990). UEM testing took place within this window at 120 minutes post dosing, while ETB testing took place later at 180 minutes post dosing. However, cortisol levels were still significantly raised for 30mg mCPP compared to placebo at 300 minutes post dosing. In addition, the 30mg dose of the drug, but not the 15mg dose, induced negative physical symptoms (nausea) in participants, a finding which also confirms the results of previous studies (Walsh et al. 1994). Taken together, these data suggest that mCPP activated 5-HT_{2C} receptors at concentrations sufficient to exert effects on eating and emotional behaviour in the model.

Appetite ratings were attenuated for men and women by 30mg mCPP, but not by 15mg mCPP, a finding that is consistent with previous reports (Cowen et al. 1995; Smith et al. 1994a). Interestingly, the results for the SQ, showed a dose-dependent effect of mCPP, but only in women. Thus, SQs were enhanced in the first half of the test meal at both 15mg and 30mg mCPP, with an earlier onset of action at the 30mg mCPP dose. mCPP failed to significantly decrease food intake compared to placebo suggesting that VAS ratings and the SQ measure derived from UEM microstructural data may be more sensitive to the effects of mCPP (at least in women) on eating than measurement of food consumption. The apparent acceleration of satiation by mCPP in lean women in the present study is similar to that observed with the 5-HT reuptake inhibitor sibutramine in obese women (Halford et al. 2010)

and may be part of a common behavioural mechanism by which 5-HT_{2C} receptor activation reduces food intake.

Differences between men and women in eating patterns and the response to mCPP were observed. Men ate more than women and at a faster rate. While both men and women had reduced appetite ratings after mCPP only women showed enhanced satiety. These differences are unlikely to be explained by gender differences in body weight, drug distribution, or metabolism because there were no gender effects observed for mCPP-induced increases in cortisol. One possibility is that although the men experienced an effect of mCPP on appetite they are less sensitive to the drug effects on actual food intake measured in the context of the laboratory. Several men ate close to or more than 1kg of pasta which suggests that they may have been eating larger amounts than usual. It would be informative to assess the response of men to the effects of mCPP on intake at usual meals taken in the home.

It appears unlikely that the effects of 15mg mCPP on appetite and satiety were confounded by nausea as this dose did not induce significant physical effects. However, it is possible that nausea could have confounded the effects on appetite seen at the 30mg dose of mCPP. This does not seem plausible, however, as neither appetite nor food intake correlated with physical symptoms. This interpretation is consistent with the results of a previous study in which nausea also failed to correlate with food intake or appetite ratings (Walsh et al. 1994). In addition, the pattern of effects for appetite and nausea are distinct, as 30 mCPP attenuated appetite earlier, and for a longer duration, than the induction of nausea.

On the basis of previous research (Charney et al. 1987; Kahn et al. 1990), it was predicted that mCPP might induce anxiety and or depression-like symptoms. The pattern of results

from the ETB, however, is not consistent with the induction of anxiety or depression-like responses by mCPP. Thus, there was no significant effect of mCPP administration on responses to fearful or angry facial expressions which are generally indicative of heightened anxiety (Mogg and Bradley 2002; Harmer et al. 2003). Nor were there any responses indicative of depression, such as the reduced recall of positive stimuli that has been observed after acute treatment with the CB_1 receptor antagonist rimonabant (Horder et al. 2009). Previous studies that have reported anxiogenic effects of mCPP generally administered the drug as an intravenous bolus, and so it is possible that effects on anxiety are less evident with the oral route of administration used in the present study (Kahn et al. 1990; Silverstone and Cowen 1994; Cowen et al. 1995; Anderson et al. 2002b). The lack of an mCPP-induced depressogenic or anxiogenic effect on the ETB tasks is consistent with the general absence of effects on the questionnaire based measures of anxiety and mood; thus there is internal consistency within the model.

mCPP dose dependently enhanced memory for emotional words, an effect that was independent of valance. This confirms that the ETB was capable of detecting effects of mCPP, even when testing was conducted after the predicted time for peak plasma levels and associated behavioural effects. Previous studies in rodents suggest that mCPP may impair memory function (Khaliq et al. 2012). However, studies in human volunteers have linked tryptophan depletion to memory impairment, and citalopram enhancement of serotonergic function with facilitation of memory consolidation (Hayward et al. 2005; Harmer et al. 2002). The present results are consistent with these findings and suggest that 5-HT_{2C} receptors may mediate the facilatory effects of citalopram on memory.

Previous research has reported that enhancing memory of an eating episode decreases subsequent food intake (Higgs and Donohoe 2011). Thus, it is possible that long term use of mCPP and other serotonergic drugs could enhance memories of food consumption and reduce subsequent food intake. This theory of drug induced memory enhancement as a behavioural mechanism to reduce food intake (Higgs 2002) is supported by findings with the *N*-Methyl-D-aspartate (NMDA) receptor antagonist memantine (Parsons et al. 2007; Foltin et al. 2008). This suggests that memory enhancement might play a role in enhanced satiation and could provide a novel target for future weight-loss drugs.

In conclusion, the present results show that the UEM/ETB experimental medicine model is capable of measuring drug effects on eating, satiety and mood in healthy human volunteers. For the first time, this research shows that effects of a low 15mg dose of mCPP on appetite and satiety are detectable using a UEM to measure drug-induced enhancement of within-meal satiation, which appears to be a promising marker of an efficacious anti-obesity drug. The model can also detect drug induced changes in cognitive processing. Thus, an enhancement of memory for emotional stimuli by mCPP was observed for the first time supporting a role for 5-HT_{2C} receptors in cognition. As the eating behaviour of men was highly variable, future studies will include women participants only, until experimental strategies to deal with this variability are developed.

CHAPTER 3: Study 2: Monitoring Eating Behaviour in the Laboratory: do we need to do it covertly?

3.1. Introduction

In Study 1, 26% of microstructural intake data were lost from the UEM due to participants unknowingly leaning on, or lifting the plate of pasta off the balance. Therefore, the aim of Study 2 was to assess whether participants could be made aware of the balance to reduce accidental interactions with it, without affecting their eating behaviour. In addition, an ad-libitum cookie snack session was also included to evaluate both the consumption of a pasta meal and a snack of cookies. This could provide a model to detect selective effects of manipulations on responses to foods differing in palatability and energy density in future studies.

The problem of UEM data loss during Study 1 has been reported in other studies. For example, Hubel and colleagues (2006) reported data loss due to participants manipulating a bowl of food situated on a balance. In addition, other researchers have reported excluding study data due to participants becoming aware of their UEM setup (Laessle et al. 2001). The UEM was designed as a covert measurement method (Kissileff et al. 1980), and has been consistently used in this manner by the majority of researchers for over three decades. However, Westerterp-Plantenga and colleagues (1991) reported that in a previous study, UEM awareness did not influence participants' intake of a lunch (Westerterp-Plantenga et al. 1990). However, in the Westerterp-Plantenga study (1990), fixed portions of food were provided to participants, which may have limited the ability to detect differences between groups. In addition, awareness of the UEM was only measured using a single food item (pasta).

It may be that awareness of being monitored by a UEM has an effect on consumption of certain foods but not others. For instance, there is evidence that observing participants while they are eating cookies reduces intake (Roth et al. 2001). In addition, if participants are not observed, but are told that the experimenter will know how many cookies they have eaten, a reduction in consumption is evident (Polivy et al. 1986). The key factor in most of these cases is often a desire to make a good impression on another individual, known as impression management (Herman, Roth and Polivy, 2003). Thus, actual or expected monitoring of food intake can reduce consumption, and it may be that eating energy dense or forbidden foods (e.g. cookies) is more susceptible to these effects, than staple foods (e.g. pasta),

To explore this possibility, ad-libitum access to cookies and pasta was provided in Study 2. There is evidence that classifying the same food item as either a snack or a meal can influence intake with the snack classification enhancing intake (Capaldi et al. 2006). In addition, it has been reported that that obese individuals are more likely to snack than lean individuals (Berteus-Forslund et al. 2005) and that unhealthy snacking can impede weight-loss (Kong et al. 2011). Therefore, ad-libitum cookies were added as a 'snack session' as this type of eating is associated with weight gain and obesity.

The aim of the present study was to test whether explicit awareness of the UEM would affect intake of a lunch of pasta and/or a snack of cookies (more energy dense and palatable). Participants were given access to ad-libitum pasta and tomato-sauce, followed twenty minutes later by access to ad-libitum chocolate chip cookies. Participants in the aware condition were made explicitly aware of the UEM, while those in the non-aware condition were not told about the UEM. It was predicted that awareness of the UEM might decrease the consumption of pasta and cookies, having a larger effect on the latter.

3.2. Methods and Materials

Participants

A total of 72 healthy women student volunteers were recruited from the School of Psychology at the University of Birmingham. During testing, 3 participants in the non-aware condition became aware of the UEM, while 30 participants accidentally interacted with the balance during their test session. Therefore, 39 participants successfully completed testing and their data were used in the analysis. The 39 participants had a mean age of 19.67 years (SD 1.24) and a mean body mass index (BMI) of 21.84 (SD 2.17). Reimbursement for participation in the study took the form of course credits or a £10 cash payment. Informed consent was obtained from participants and ethical approval was provided by the University of Birmingham Research Ethics Committee. The study was conducted in accordance with Good Clinical Practice and the ethical standards laid down in the 1964 Declaration of Helsinki. Participants were not recruited if they: had food allergies; smoked cigarettes; took medication that affected appetite; were diabetic or had participated in a previous study using a UEM. All of these were assessed via questionnaire in the laboratory.

Design

A between-subjects design was used with a single factor of awareness with two levels: aware versus non-aware. Participants were randomly allocated to one of these conditions and order of testing within sessions was counterbalanced so that half of the participants completed a batch of questionnaires followed by a computer task, while the other half had the order reversed. As Yeomans (1996) had reported significant effects with 18 participants per group in a between-subjects design, the aim was to recruit at least 18 participants to each group.

Universal Eating Monitor (UEM)

Food was served on a UEM (Sussex Ingestion Pattern Monitor; Sartorius Model CP4201; Sartorius Ltd, Epsom, UK; 0.1g accuracy). The UEM was connected to a computer, and relayed balance weights every 2 seconds (refer to Study 1 for a more detailed description).

Lunch: The pasta was prepared and served in the same manner as Study 1, however, the total amount of pasta served was increased to 220g (253 calories per 220g serving). This was necessary, as some participants consume less of the pasta sauce than others, which if left on the dish in sufficient amounts (e.g. >50g), prevents the trigger for the automatic refill. Hence, the total amount was increased to eliminate this occurrence. All other aspects of the pasta meal were the same as for Study 1.

Snack: Bowls were filled with 80g of Maryland chocolate chip cookies (390 calories per 80g serving; purchased from Sainsbury UK; refer to Figure 3.1). Each cookie was broken into 6-7 pieces to reduce the likelihood that participants could track the number of cookies they ate, an approach used in previous research (Higgs and Woodward, 2009). The amount of cookies served ensured that participants were provided with more of the snack than they were likely to consume (Higgs and Woodward, 2009). The bowl was set on a placemat, and each time a participant ate 10g of cookies, the UEM software interrupted the participant to complete VAS ratings as described above for the pasta meal. After consuming 60g of cookies, the participant was interrupted and provided with a fresh bowl of 80g of cookies. Participants were asked to eat until they felt 'comfortably full'.

Figure 3.1 Left panel shows a standard 80g serving of chocolate chip cookie pieces on the UEM setup. Right panels show the UEM setup (upper) and a serving of the tomato-based pasta on the UEM setup (lower).

Stop Signal Reaction Time Task (SSRT)

Impulsivity has been reported to affect the consumption of food (Guerrieri et al, 2007), hence, the SSRT was included to assess differences between groups on this measure. The SSRT (as described in Verbruggen et al. 2008) involves presenting participants with either a square or a circle shape on a screen that they are required to identify. On no-signal trials, a shape is presented and participants respond by identifying the shape. On stop-signal trials, an auditory stop signal alerts participants to withhold making a response to the presentation of the shape. The task consists of 32 practice trials followed by 192 experimental trials and takes 20 minutes. Calculation of the stop signal reaction time provides a measure of inhibition of response (behavioural impulsivity).

Procedure

Participants arrived in a pre-meal state having refrained from eating for 2 hours prior to arrival. They completed a consent form, and were screened using a lifestyle questionnaire which collected demographic information. After this, they completed the stop signal reaction time task as a measure of response inhibition and a series of questionnaires. The questionnaires comprised the Barratt Impulsivity Scale (BIS 11– Patton et al. 1995) and the Behavioural Inhibition/Approach Scales (BIS/BAS - Carver and White, 1994) as additional measures of impulsivity. Participants also completed the TFEQ, as a measure of dietary restraint, and the Power of food Scale (PFS, Lowe et al. 2009), as a measure of sensitivity to food to ensure no differences between groups. A breakfast questionnaire was used to ensure that no food was eaten within the previous two hours, and participants completed a set of baseline VAS for rated mood and appetite on a scale from 0-100mm (0mm anchor = not at all, 100mm anchor = extremely): 'alertness'; 'disgust'; 'drowsiness'; 'light-headed'; 'anxiety'; 'happiness'; 'nausea; 'sadness'; 'withdrawn'; 'faint'; 'hungry'; 'full'; 'desire to eat' and 'thirst'.

Participants were taken to a room containing the UEM. Those in the aware condition were shown that there was a balance underneath the table. They were told that the balance would record the weight of their bowl and food as they ate during the meal and that this information would be stored on the computer it was connected to for later analysis. Those in the non-aware condition were not given this information. After they had been given instructions regarding the procedure (i.e. that they could eat as many bowls of pasta as they wished until they were comfortably full), the participants were asked to eat lunch, as described above. After they had finished, they immediately completed another set of VAS, and were given a 20 minute rest period in another room, where they were offered a home furniture magazine to

read. They then completed another set of VAS directly before being taken back to the UEM to eat the ad-libitum snack of cookies. Following this snack, participants completed a final set of VAS, and had their height and weight taken for BMI calculation. To assess awareness of the UEM they were then asked what they thought the study was about, and whether they had noticed the balance at any point, or whether they thought their intake was being recorded during the study. After this, participants were debriefed, thanked for their time, and compensated with course credits or a cash payment.

Data Analysis

General: Effects of awareness were determined with independent t-tests. Repeated measures analysis of variance (ANOVA) was used to examine temporal effects and interactions with awareness. Only significant effects of awareness, or temporal interactions with awareness, were followed up with planned comparisons and all post-hoc t-tests used the Bonferroni correction. Violations of sphericity were addressed using the Greenhouse-Geisser correction.

VAS: To establish a factor structure for the VAS, a principal components analysis (PCA) was run with varimax rotation. Analysis of the 14 items provided 3 factors with eigenvalues > 1, accounting for 59.99% of the variance. Items that loaded > 0.5 onto a factor were included, resulting in three factors of 3 or more items: appetite (hunger, fullness and desire to eat); negative effects (sadness, nausea, disgust, faint, withdrawn, lightheaded) and arousal (alertness, happiness, drowsiness). Scores for each of the factors were calculated by summing the scores for all items in that factor, and then dividing by the number of items. Items with a negative scale, were inverted to match the other items. Anxiety and thirst did not load onto these factors and were analysed separately.

UEM: The following measures were calculated for UEM data: amount eaten, time spent eating, eating rate and pause between mouthfuls. The first three measures are standard measures of eating behaviour in microstructural studies, while the latter is a novel measure. It was included because it provides data on the time taken between mouthfuls, which may be a useful indication of motivation to eat. The pause measure also provides useful data on frequency of mouthfuls. For instance, if time is constant, then shorter pauses equate to more mouthfuls and vice versa. The data for each measure was also divided into quartiles for analysis (effects of quartile were not investigated further).

3.3. Results

Baseline Measures and Visual Analogue Scales

To ensure there were no group differences in demographics and behaviours which might affect food consumption (e.g. impulsivity, food sensitivity, cognitive restraint, etc.) all scores were analysed using independent t-tests comparing aware and non-aware conditions. There were no significant differences for all scales and subscales: BMI; Age; TFEQ; BIS 11; PFS; BIS; BAS; and SSRT (all $p > 0.05$ – Table 3.1).

Table 3.1 Mean baseline scores for non-aware and aware groups (standard error of the mean)

Measure	Non-aware	Aware
BMI	21.9 (0.5)	21.8 (0.5)
Age	20.0 (0.4)	19.4 (0.2)
TFEQ Cognitive Restraint	8.3 (1.0)	9.1 (1.2)
TFEQ Disinhibition	8.8 (0.7)	7.1 (0.8)
TFEQ Hunger	7.3 (0.7)	7.2 (0.7)
BIS 11	69.1 (2.7)	66.6 (2.1)
PFS	40.8 (2.5)	43.7 (2.4)
BIS	23.2 (1.0)	24.1 (0.6)
BAS Drive	10.4 (0.5)	11.0 (0.5)
BAS Fun seeking	12.0 (0.4)	11.7 (0.5)
BAS Reward Responsiveness	16.7 (0.4)	17.4 (0.4)
SSRT (milliseconds)	233.0 (7.0)	233.8 (5.0)

BMI - Body Mass Index; TFEQ - Three Factor Eating Questionnaire; BIS 11 - Barratt Impulsiveness Scale; PFS - Power of Food Scale; BIS - Behavioural Inhibition Scale; BAS - Behavioural Activation Scale; SSRT - Stop Signal Reaction Time

VAS data were analysed by condition (aware versus non-aware) and by time (pre-pasta, post-pasta, pre-cookies and post-cookies). For appetite, arousal, anxiety and thirst there were main effects of time (all $p < 0.01$ – means displayed in Table 3.2) which were not analysed further, but there were no effects of condition (see Table 3.3 for means) and no interactions (all $p > 0.05$). For negative effects, there was a main effect of time (F (3 99) = 18.48; $p < 0.001$), no effect of condition (F (1 33) = 0.10; $p > 0.05$) and a significant interaction between condition and time (F (3 99) = 3.36; $p < 0.05$). T-tests comparing negative effects between conditions for each time point did not show any significant differences (all $p > 0.05$); baseline difference (pre-pasta) for the non-aware versus aware was the closest to significance (18.06mm versus 12.22mm, respectively; t (37) 1.29, $p = 0.2$).

Table 3.2 Mean VAS ratings over time (standard error of the mean)

VAS Measure	Pre-Pasta	Post-Pasta	Pre-Cookies	Post-Cookies
Appetite	72.5 (2.6)	15.3 (2.0)	17.2 (2.2)	9.7 (1.7)
Negative Effects	15.1 (2.3)	9.7 (1.5)	8.8 (1.4)	7.6 (1.1)
Anxiety	21.3 (3.5)	11.4 (2.3)	9.1 (1.6)	8.7 (1.7)
Arousal	53.7 (2.5)	62.1 (2.4)	57.6 (2.1)	59.5 (2.1)
Thirst	53.1 (4.4)	43.7 (3.8)	37.0 (4.2)	33.5 (4.2)

Table 3.3 Mean VAS Measures separated by condition (standard error of the mean)

VAS Measure	Non-aware	Aware
Appetite	27.5 (2.1)	30.6 (2.1)
Negative Effects	10.8 (2.3)	9.7 (2.3)
Anxiety	12.9 (3.2)	13.2 (3.1)
Arousal	60.6 (2.7)	55.8 (2.6)
Thirst	42.3 (5.5)	42.7 (5.4)

Universal Eating Monitor

Two-factor repeated-measures ANOVA with the within-subjects factor quartile (quartiles 1, 2, 3 and 4) and the between-subjects factor awareness (aware versus non-aware) were used to analyse the following measures: total amount eaten; time spent eating; pause between mouthfuls and eating rate.

Pasta Lunch: There was a significant effect of quartile (both $p < 0.05$) but no effect of awareness and no significant interaction (all $p < 0.05$) for amount eaten, time spent eating and pause between mouthfuls (Table 3.4). There were there were no significant effects or interactions for eating rate (all $p > 0.05$).

Cookie Snack: There was a main effect of awareness for eating rate as participants in the aware condition ate cookies at a slower rate than those in the non-aware condition (F (1 37) = 5.70; $p < 0.05$; 10.23 versus 13.40 g/min; Table 3.4). There was no main effect of quartile or a significant interaction (both $p > 0.05$). There was a significant effect of quartile on amount eaten, time spent eating and pause between mouthfuls, (all $p < 0.05$ – Table 3.4) but no other main effects or interactions reached statistical significance (all $p < 0.05$).

Table 3.4 UEM Measures for Pasta and Cookies, split by non-aware versus aware groups and quartiles (mean with standard error of the mean in parentheses)

	Non-aware				Aware			
Measure	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Pasta								
Amount eaten (grams)	86.6 (5.1)	89.8 (5.2)	86.9 (6.4)	98.3 (5.3)	85.5 (7.8)	90.5 (8.2)	91.4 (7.6)	97.8 (8.1)
Time spent eating (seconds)	89.0 (0.6)	96.7 (13.6)	99.4 (11.5)	137.4 (36.9)	97.9 (6.3)	82.7 (5.1)	91.9 (5.7)	114.4 (9.9)
Pause between mouthfuls (secs)	8.0 (0.5)	7.8 (1.0)	7.9 (0.6)	9.8 (1.9)	9.3 (0.6)	7.5 (0.4)	8.1 (0.4)	9.6 (0.6)
Eating rate (g/min)	61.1 (4.2)	66.1 (5.5)	64.6 (7.5)	60.3 (7.0)	52.8 (3.3)	66.0 (4.7)	61.0 (4.2)	55.7 (4.7)
Cookies								
Amount eaten (grams)	7.9 (0.8)	9.98 (1.0)	10.5 (1.1)	11.9 (1.0)	7.6 (1.1)	9.1 (1.1)	9.1 (1.1)	11.0 (1.2)
Time spent eating (seconds)	39.5 (4.2)	42.4 (4.0)	61.7 (9.0)	83.5 (15.5)	46.3 (6.1)	65.8 (7.1)	62.2 (7.5)	100.2 (14.2)
Pause between mouthfuls (secs)	9.6 (1.1)	9.6 (1.5)	12.5 (2.7)	17.8 (5.4)	14.3 (1.7)	16.1 (1.9)	15.6 (1.6)	19.1 (2.7)
Eating rate (g/min)	14.0 (1.8)	14.7 (1.1)	12.6 (1.3)	12.4 (1.7)	11.7 (1.5)	9.5 (1.2)	10.9 (1.5)	8.8 (1.2)

Computerised within-meal VAS

VAS ratings made during the meal, were used to calculate the mean rating for each quartile of the meal. These were analysed using two-factor repeated-measures ANOVA, with the factors of quartile (within-subject) and condition (between-subject).

Pasta: For the hunger VAS, there was a main effect of quartile (F (3, 86) = 150.44; $p < 0.001$), and a trend for an effect of condition, with greater hunger in the aware versus non-aware group when collapsing across quartiles (F (1 37) = 3.17; $p = 0.08$; 42.31 versus 35.01mm, respectively); but no significant interaction ($p > 0.05$). For fullness there was a

main effect of quartile (F (3 111) = 145.08; $p < 0.001$), and for condition, with lower fullness in the aware versus non-aware condition (55.07 versus 62.57; F (1 37) = 3.17; $p < 0.05$; Figure 3.2) but no significant interaction ($p > 0.05$). Finally, for the pleasantness ratings, there was a main effect of quartile (F (3 111) = 23.62; $p < 0.001$), but no effect of awareness, or a significant interaction (both $p > 0.05$).

Cookies: For rated hunger, there was a main effect of quartile (F (3 111) = 6.97; $p < 0.001$), and a main effect of awareness with greater hunger in the aware group versus non-aware (18.39 versus 9.06; F (1 37) = 6.16; $p < 0.05$; Figure 3.2), but no interaction (F (3 111) = 0.41; $p > 0.05$). For the fullness VAS, there was a main effect of quartile (F (3 111) = 11.24; $p < 0.001$), no effect of condition (F (1 37) = 0.68; $p > 0.05$), but a significant interaction between quartile and condition (F (3 111) = 2.80; $p < 0.05$); t-tests did not reveal any significant differences between the aware and non-aware group, for any quartile. For the pleasantness VAS, there was no effect of quartile, condition, or a significant interaction (all p > 0.05.

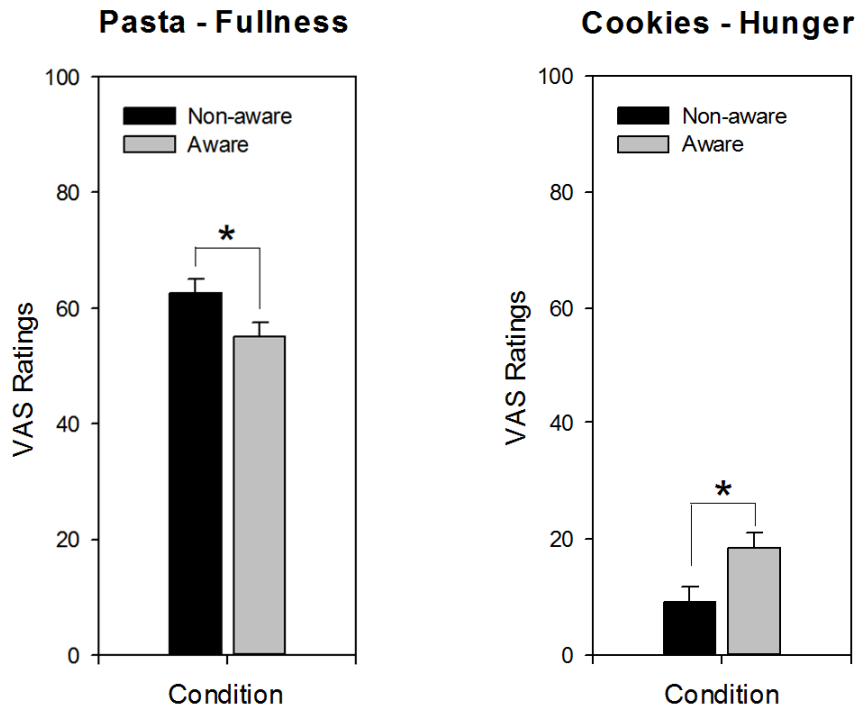

Figure 3.2 Mean fullness and hunger ratings whilst consuming pasta and cookies, respectively. Rated fullness whilst eating pasta (left) was significantly decreased when participants were aware (versus non-aware); mean rated hunger whilst eating cookies (right) was significantly increased when participants were aware. * $p < 0.05$

Satiation Quotient

Satiation quotients were calculated (refer to Study 1) and analysed. For pasta, there was a main effect of quartile (F (2 78) = 3.85; $p < 0.05$), but no effect of condition, nor an interaction (both p > 0.05). For cookies, there a main effect of condition, whereby participants in the aware group showed significantly lower satiation scores compared to the non-aware group (0.16 versus 0.44; (F (1 37) = 4.77; $p < 0.05$). There was no effect of quartile or interaction (both $p > 0.05$).

3.4. Discussion

Awareness of the UEM did not affect the total amount of pasta or cookies consumed. It also had no significant effect on the time taken to consume the food, or the pauses taken between mouthfuls. Awareness of the UEM did not affect the eating rate of pasta, however, it

significantly reduced the eating rate of cookies. Calculation of the satiation quotient showed that awareness did not affect the satiating capacity of the pasta, but weakened the effect of the cookies. Hence, awareness had a limited effect on eating behaviour. Overall, the effects of awareness are confined to the cookie snack, but do not affect total consumption.

The decrease in cookie eating rate but not pasta eating rate supports the suggestion that the effects of awareness may be related to the type of food consumed. Thus, consumption of highly palatable, energy dense foods may be more susceptible to awareness of being monitored. As more energy dense foods such as cookies are often perceived as "unhealthy" and "dangerous" (Macht et al. 2003) and their consumption can be interpreted as a negative eating behaviour (Stevenson et al. 2007), it is plausible that consuming cookies was viewed by participants as a less acceptable behaviour than consuming the pasta. Therefore, as individuals are motivated to present themselves in a positive manner in an eating situation (Herman et al. 2003), individuals who were aware of the UEM might have reduced their rate of eating cookies, but not the pasta, to present a more positive social image of themselves. While it might be hypothesised that participants would have reduced their overall intake of cookies as well as eating rate, the presentation of cookies in small pieces could have made it difficult for participants to monitor and limit their intake. This suggestion is supported by evidence that participants eat more of the same food when it is presented amorphously, as numerous small pieces/parts, than as a whole item (Chang et al. 2012).

The limited effects of awareness of the UEM on eating behaviour appear insufficient to justify continued use of a covert approach, which is associated with data loss (Study 1; Laessle et al. 2001; Hubel et al. 2006). It is simpler to ensure that participants remain aware, rather than non-aware of the UEM during test sessions. Once informed of the UEM, it is highly unlikely that participants will forget its presence during the test session. However, in a

non-aware study, participants may not remain non-aware, and some participants who become aware will not admit this during debrief due to demand characteristics. Therefore, an aware approach may ensure a more consistent experience for participants, and by extension, more robust data.

The limited, but nonetheless selective decrease in the rate of cookie consumption reinforces the importance of assessing a range of food types. In addition, the observation that participants consumed a significant amount of cookies (36.7grams; approximately 4 cookies; 179 calories) soon after lunch suggests that the UEM can be used to study the phenomenon of eating in the absence of hunger. This type of eating is associated with hedonically driven consumption of food and is more pronounced in overweight individuals (Shomaker et al. 2010). This experimental design may therefore offer an opportunity to examine the potential behavioural mechanisms of anti-obesity drugs that reduce the hedonic value of food eaten in the absence of hunger.

To conclude, the data from Study 2 suggest that the UEM may be used to assess eating behaviour without the need for participants to be unaware of its presence. In addition, the inclusion of a snack session as part of the UEM model may allow for testing of selective effects of drugs on hedonically driven eating. For future work with the model, it will be necessary to test other populations of interest, such as obese participants, where awareness could potentially interact with factors such as body image and self-esteem. More immediately, a direct test of whether awareness affects responses during a drug study, and whether an appetite suppressing drug has a selective effect within this setup, would help provide further data for the validity of a non-covert approach, and for the addition of a snack session (Study 6).

CHAPTER 4: Studies 3 and 4: The P1vital® Oxford Emotional Test Battery (ETB): does practice or satiety affect measures of cognition and emotional processing?

4.1. Introduction

To date, the ETB has been used in between-subjects designs (e.g. Murphy et al. 2008; Harmer et al. 2009) and has not been tested for repeated use. In many instances, cross-over paradigms are preferable, as they can control for individual differences, particularly with regard to eating behaviour. In addition, the effects of eating food prior to ETB testing are unknown but are important to determine for future ETB studies involving drugs that affect appetite. Therefore, the studies presented in this chapter investigated the influence of repeated testing and satiation on ETB performance.

The aim of Study 3 was to investigate whether practice effects occur for any of the ETB tasks by testing the entire battery repeatedly. It is well documented that practice effects occur for many cognitive tasks (Hausknecht et al. 2007; Benedict and Zgaljardic, 1998). However, for some tasks, these effects plateau quickly and a stable level of performance is reached (Bartels et al. 2010). The factors that mediate this process probably relate to the cognitive function under investigation, the type of task being used, and the complexity of the task (Benedict and Zgaljardic, 1998). For instance, practice effects are more likely to occur for more complex tasks before stabilising (Falleti et al, 2006). Healthy women volunteers were tested on four occasions, with each test session separated by a 7 day interval; 7 days is a standard wash out interval in many crossover drug studies. Responses were compared across sessions to examine whether any practice effects occurred. Based on previous research (Benedict and Zgaljardic, 1998; Falleti et al, 2006), it was hypothesised that practice effects would occur for

the more complex tasks such as the facial expression recognition task and emotional recall task.

The aim of Study 4 was to assess the effects of satiation on ETB performance. Much of the previous work investigating the effects of food intake on measures of cognition and mood has concentrated on the effects of meal composition on performance. For instance, ingesting a lunch high in protein or carbohydrates decreases mood, and produces selective impairments in different aspects of attention (Smith et al. 1988). Lunches with higher than usual levels of fat or carbohydrate also impair mood and cognitive efficiency (Lloyd et al.1994). In addition, large lunches are associated with more errors on attentional tasks (Smith et al. 1991). A number of studies have compared the performance of fasted versus fed participants, showing that consumption of breakfast can improve cognitive performance on memory tasks (Benton and Parker, 1998; Smith et al. 1994b), and that consumption of palatable snack foods can enhance mood (Macht and Dettmer, 2006). However, these results are likely to be influenced by time of eating (breakfast versus lunch) and the palatability of the food consumed. In Study 4 the effects of satiating participants before ETB testing was examined. It was hypothesised that participants satiated with lunch would show impaired cognitive performance, and possibly, a decrease in mood, compared to those who were not given lunch.

4.2. Study 3 Overview and Design

Study 3 tested whether repeated sessions on the ETB led to practice effects. A within-subjects design was used, with a single factor of session comprised of four levels: session 1; session 2; session 3; and session 4. Each session was run at the same time of day, one week apart and participants completed the ETB at all four sessions. The order of completing questionnaires and the ETB during sessions was counterbalanced across participants; half of the participants

always completed the questionnaires followed by the ETB, while the other half were tested in the reverse order each time.

4.3. Study 3 Methods and Materials

Participants

30 healthy women student volunteers (mean age = 18.9 years; mean BMI = 21.48; mean NART score = 111) were recruited for the study from the University of Birmingham. Informed consent was obtained and participants were given either £20 cash or course credits upon completion. The study was approved by the University of Birmingham Research Ethics Committee and was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Participants were excluded from the study if they were under 18 or over 65 years of age and if they were not fluent English speakers as determined by the NART. Using a screening questionnaire, participants were excluded if they: had previously taken part in an ETB study; were dyslexic; smokers; taking medication; had consumed a high amount of caffeine (> 750mg; Winston et al. 2005) or alcohol (> 3 units; NICE, 2010) in the last 24 hours; or had current or past depression determined by use of the SCID.

P1vital® Oxford Emotional Test Battery (ETB)

The ETB is a computerised battery that comprises five validated cognitive tests (see www.p1vital.com), which has been used in previous drug studies (e.g. Harmer et al. 2004; Murphy et al. 2008; refer to Study 1 for full details).

Procedure

Participants completed a consent form before completing the screening measures. They had their height and weight taken for BMI calculation and then completed the NART, the SCID

(questions relating to depression only), a lifestyle questionnaire and an alcohol and caffeine questionnaire (documenting intake in the last 24 hours). Participants were then given VAS with the following mood and appetite items to rate on a scale from 0-100mm (0mm anchor = not at all, 100mm anchor = extremely): 'alertness'; 'disgust'; 'drowsiness'; 'light-headed'; 'anxiety'; 'happiness'; 'nausea'; 'sadness'; 'withdrawn'; 'faint'; 'hungry'; 'full'; 'desire to eat' and 'thirst'. After this, participants completed the ETB (which took approximately 60 minutes) and then the TFEQ and the BDI questionnaire pack in a counterbalanced order. Finally, participants completed another VAS questionnaire.

Participants returned for three further sessions, which were seven days apart from one another, and always at the same time of day. The procedure above was repeated for each session with the exception of: consent, BMI measurement, NART, SCID and the lifestyle questionnaire. On completing their last session, participants were debriefed, thanked for their time and compensated with either £20 cash or course credits.

Data Analysis

General: Within-subjects analysis of variance (ANOVA) was used to analyse the data. Bonferroni correction was used for all post-hoc t-tests and violations of sphericity were addressed using Greenhouse-Geisser correction.

VAS: To establish a factor structure for the VAS, a principal components analysis (PCA) was run with varimax rotation. Analysis of the 14 items provided 4 factors with eigenvalues > 1, accounting for 66.64% of the variance. Items that loaded > 0.5 onto a factor were included, resulting in 4 factors of 3 or more items: appetite (desire to eat, hungry, fullness and thirst); negative physical effects (faint, lightheaded and nausea); arousal (alertness, happiness and

drowsiness); negative mood (anxiety, sadness and disgust). Withdrawn did not load > 0.5 onto any of the factors and was analysed separately. Scores for each of the factors were calculated by summing the scores for all items in that factor and then dividing by the number of items. Items with a negative scale, were inverted to match the other items.

ETB Data: Task specific effects that were relevant to the task but not to the experimental manipulation are presented to confirm the ability to detect effects of emotion and or valence. For main effects of emotion, t-tests were used to further analyse the data. Main effects of session and interactions between session and emotion or valence were also analysed.

4.4. Study 3 Results

Subjective Measures

Questionnaires: ANOVA of scores from the BDI, TFEQ subscales (cognitive restraint, disinhibition and hunger) and the Alcohol and Caffeine questionnaire (ACQ) revealed no significant differences across the four test sessions, (all $p > 0.05$ – Table 4.1).

Visual Analogue Scales (VAS): VAS scores were analysed with ANOVA using the factors of session (session 1, session 2, session 3 and session 4) and time (pre ETB and post ETB). For appetite, negative physical effects, negative mood and withdrawn, there were no main effects or interactions (all $p > 0.05$). However, for arousal, there were main effects of session (F (3 87) = 3.12; $p < 0.05$) and time (pre ETB = 63.34mm versus post ETB = 55.64mm; F (1 29) = 19.54; $p < 0.001$) but no significant interaction (F (3 87) = 0.52; $p > 0.05$). Following up the main effect of session with t-tests, none of the comparisons were significantly different, though the closest to significance was the decrease in arousal from session 1 to session 3 (t (29) 2.70; $p = 0.07$ – Table 4.1).

Table 4.1 Questionnaires and Visual Analogue Scale mean scores (standard error of the mean). VAS scores are averaged over each test session.

	Session			
Measure	1	2	3	4
BDI	7.3 (1.0)	7.1 (1.1)	7.2 (1.3)	6.9 (1.3)
TFEQ Cognitive Restraint	7.7 (1.1)	7.0 (1.1)	7.1 (1.2)	6.8 (1.2)
TFEQ Disinhibition	7.1 (0.7)	7.3 (0.6)	7.4 (0.7)	7.3 (0.7)
TFEQ Hunger	6.1 (0.5)	6.7 (0.6)	6.8 (0.7)	6.4 (0.6)
Alcohol (units)	0.1 (0.1)	0.0 (0.0)	0.1 (0.0)	0.0 (0.0)
Caffeine (mg)	186.5 (18.2)	179.7 (22.8)	207.0 (32.1)	175.7 (26.3)
VAS Appetite	45.8 (1.4)	44.6 (1.3)	45.5 (1.5)	43.2 (1.4)
VAS Negative Physical Effects	6.0 (1.3)	7.0 (1.8)	5.4 (1.2)	5.0 (1.2)
VAS Negative mood	7.2 (1.1)	8.1 (1.4)	9.3 (1.9)	7.7 (1.6)
VAS Withdrawn	7.5 (1.7)	6.6 (1.6)	7.0 (1.6)	8.4 (2.0)
VAS Arousal	64.1 (2.7)	57.6 (2.8)	57.0 (2.9)	59.3 (3.1)

ETB Data

Emotional categorisation task (ECAT): Accuracy data showed a main effect of valence with negative words categorised more accurately than positive words (F (1 26) = 8.14; $p < 0.01$; see Table 4.2). However, there was no effect of session nor an interaction between valence and session (both $p > 0.05$). For reaction time, there was no effect of valence, session, nor an interaction between valence and session (all $p > 0.05$).

Emotional recognition memory task (EMEM): There was a main effect of valence for accuracy (F (1 29) = 80.33; $p < 0.001$) and for reaction time (F (1 29) = 21.68; $p < 0.001$) (see Table 4.2). However, there were no effects of session, nor an interaction between valence and session, either for accuracy or reaction time (all $p > 0.05$). For response bias,

there was an effect of valence (positive words = -0.138 versus negative words = 0.371; F (1 28) = 140.99; $p < 0.001$ – see Table 4.2), but no effect of session, nor an interaction (both $p > 0.05$).

Table 4.2 ECAT Accuracy and EMEM Accuracy, Reaction Time and Response Bias scores, split by valence (standard error of the mean)

		Valence	
ETB Task	Measure	Positive	Negative
Emotional Categorisation	Accuracy	92.6 (1.4)	94.8 (1.0)**
Emotional Recognition	Accuracy	82.6 (1.9)	67.7 (2.3)***
	Reaction Time	964.0 (43.5)	1086.2 (53.3)***
	Response Bias	-0.14 (0.06)	0.37 (0.05)***

**$p < 0.01$; ***$p < 0.001$

Emotional recall task (EREC): There were main effects of valence for the number of words correctly recalled (negative words = 8.03 versus positive words 9.93; F (1 29) = 16.36; $p < 0.001$), and a main effect of session (F (3 87) = 44.66; $p < 0.001$ – Figure 4.1), but no significant interaction between valence and session (F (3 87) = 1.40; $p > 0.05$). Examining the effect of session, all sessions were found to be significantly different from one another (with the exception of session 3 versus session 4), with accuracy increasing over sessions (all $p < 0.01$ - Figure 4.1). For the number of incorrectly recalled words, there was a main effect of valence (F (1 29) = 7.42; $p < 0.05$), a main effect of session (F (3 87) = 8.21; $p < 0.001$), and an interaction between session and valence (F (2 70) = 3.84; $p < 0.05$). Breaking down the interaction by emotion, there was no effect of session for incorrectly recalled negative words (F (3 87) = 0.61; $p > 0.05$), but there was an effect of session for incorrectly recalled positive words (F (2 67) = 9.43; $p < 0.001$ – Figure 4.1). T-tests showed significant decreases in falsely recalled positive words from session 1 (2.60 words) to session 3 and session 4 (1.30

and 1.07 words: t (29) 4.04; $p < 0.01$; t (29) 3.89; $p < 0.01$ – Figure 4.1). There was also a trend for a decrease from session 1 to session 2 (2.60 to 1.77 words; t (29) 2.71; $p = 0.07$).

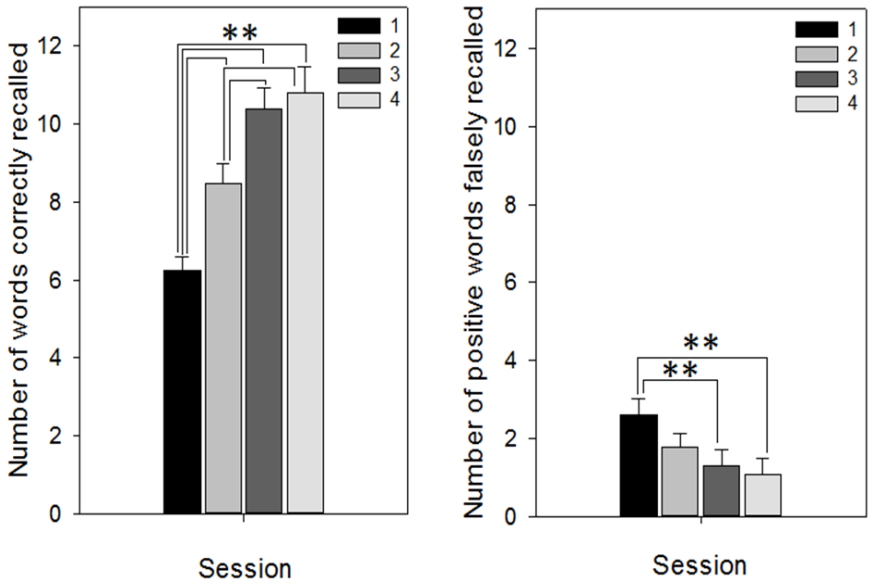

Figure. 4.1 Emotional recall task (EREC): Correctly recalled words (**left**) and falsely recalled positive words (**right**), for the four test sessions. Number of words correctly recalled increased across each session, with the exception of session 3 to session 4. For positive words, there was a significant decrease from session 1 to sessions 3 and 4. Error bars represent standard error of the mean. **$p < 0.01$

Facial expression recognition task (FERT): There were main effects of emotion (F (3 98) = 18.88; $p < 0.001$) and session (F (3 87) = 6.44; $p < 0.01$) on accuracy, but no significant interactions ($p > 0.05$). T-tests on the effect of emotion showed that facial expressions of anger, disgust, fear, sadness and surprise were categorised significantly less accurately than neutral faces (all $p < 0.01$), while accuracy for happy faces was not significantly different from neutral faces ($p > 0.05$) (see Table 4.3). Accuracy was significantly higher during session 2 and session 4 (57.90% and 58.83%) than session 1 (55.27% - Figure 4.2) (t (29) -3.16; $p < 0.05$; t (29) -4.39; $p < 0.01$).

There were main effects of emotion (F (4 102) = 32.87; $p < 0.001$) and session (F (3 84) = 6.30; $p < 0.01$) for reaction time but no interaction ($p > 0.05$). For the effect of emotion, reaction times to anger, disgust, fear, sadness and surprise expressions were significantly slower than to neutral faces (all $p < 0.01$) (see Table 4.3), while reaction times to happy faces and neutral faces did not differ ($p > 0.05$) (see Table 4.3). Reaction times were significantly different between all sessions (with the exception of session 3 versus session 4), with reaction times decreasing over sessions (Figure 4.2 - all $p < 0.01$).

For response bias, there was a main effect of emotion (F (3 100) = 96.02; $p < 0.001$), a marginal effect of session (F (2 69) = 2.94; $p = 0.05$) and a trend towards a significant interaction between emotion and session (F (5 135) = 2.26; $p = 0.06$). For the effect of emotion, participants were more biased to all emotional faces compared to neutral expressions (all $p < 0.001$ – Table 4.3). Breaking down the interaction by emotion, there was only an effect of session for surprise (F (3 87) = 3.30; $p < 0.05$). Comparisons between sessions showed a trend towards an increase in response bias from session 1 (0.704) to sessions 2, 3 and 4 (7.16, 7.61, and 7.60), however, these differences were not statistically significant (all $p > 0.05$).

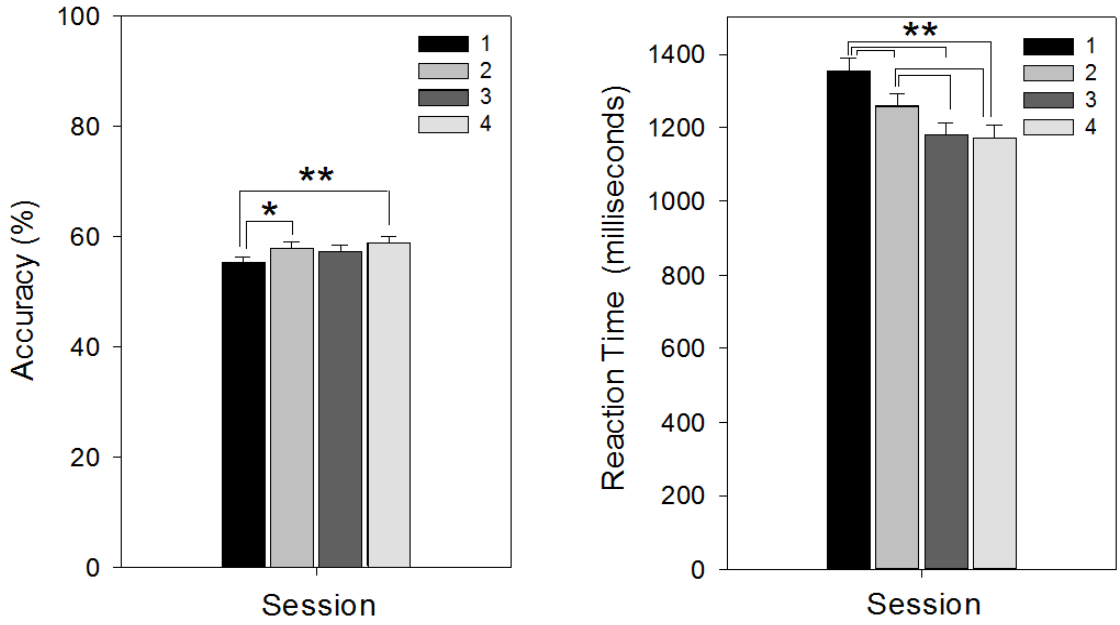

Figure. 4.2 Facial expression recognition task (FERT): mean accuracy (**left**), and mean reaction time (**right**), averaged over all emotions, for the four test sessions. Accuracy increased from session 1 to session 2 and session 4, while reaction time decreased across each session, with the exception of session 3 to session 4. Error bars represent standard error of the mean. *$p < 0.05$; **$p < 0.01$

Table 4.3 FERT Accuracy, Reaction Time, and Response Bias scores, for each emotion vs. neutral faces (standard error of the mean)

Emotion	Reaction Time	Accuracy	Response Bias
Anger	1335.7 (57.1)***	45.4 (2.4)***	0.7 (0.0)***
Disgust	1244.2 (44.9)***	52.4 (2.2)***	0.7 (0.0)***
Fear	1483.2 (48.5)***	50.4 (2.5)***	0.7 (0.0)***
Happy	1060.5 (15.8)	69.2 (1.4)	0.9 (0.0)***
Neutral	1076.9 (30.0)	71.2 (2.7)	-0.1 (0.0)
Sad	1216.9 (35.5)**	53.7 (2.8)**	0.7 (0.0)***
Surprise	1257.8 (34.3)***	59.2 (1.6)***	0.7 (0.0)***

**$p < 0.01$; ***$p < 0.001$

Faces dot probe task (FDOT): There was a main effect of masking (masked faces= 96.23% versus unmasked faces = 95.25%; (F (1 29) = 7.40; $p < 0.05$) on accuracy, but no effect of session, emotion (fear versus happy) or congruence (congruent versus incongruent probe location) nor any interactions (all $p > 0.05$). For reaction time, there was a main effect of session (F (3 87) = 6.11; $p < 0.01$), whereby reaction times significantly decreased from session 1 to session 4 (616.32ms to 579.39ms - t (29) 4.73; $p < 0.001$) (Figure 4.3). No other main effects or interactions were observed for reaction time (all $p > 0.05$).

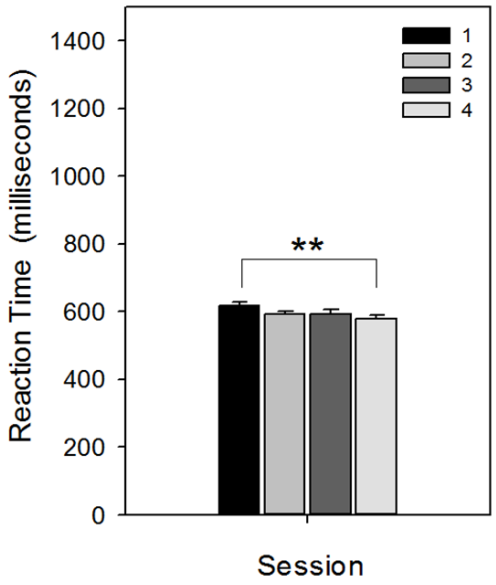

Figure. 4.3 Faces dot probe task (FDOT): Mean reaction times, averaged over both emotions, for the four test sessions; reaction time decreased significantly from session 1 to session 4. Error bars represent standard error of the mean. **$p < 0.01$

4.5. Study 3 Discussion

Repeated testing with the ETB revealed that the emotional categorisation task (ECAT) and emotional memory recognition task (EMEM) were free from practice effects. Performance on the facial expression recognition task (FERT), faces dot probe task (FDOT) and emotional recall task (EREC) was subject to practice effects; however, these effects plateaued before the final test session.

For the ECAT, it is perhaps not surprising that accuracy was stable (i.e. individuals consistently liked and disliked to be described by positive and negative words, respectively), because self-concept is relatively stable over time (Demo, 1992). While it might have been expected that participants would have become faster at selecting these descriptors over repeated sessions, evidence shows that self-referent stimuli are processed automatically and faster than non-self-referent stimuli (Bargh, 1992; Geller and Shaver, 1976). Therefore, it is possible that participants were performing at maximal speed from the first test session. As the EMEM task also showed a lack of practice effects for accuracy and reaction times, it is possible that participants were also responding as quickly and accurately as possible on this task from the first session to the same self-referent stimuli.

For the FDOT, accuracy was high and did not change over test sessions, most likely due to the simplicity of the task and its low cognitive demand. Reaction time decreased from the first to the last session, but was stable across the last three sessions. Consequently, in future studies the first session could be a training day, and subsequent sessions could use be used for repeat testing. This is similar to the dual-baseline approach in which if there is a change between session 1 and session 2, but no changes thereafter, session 2 can be used as a baseline for comparison with subsequent sessions (McCaffrey and Westervelt, 1995).

The FERT results showed that accuracy was stable after the first session. Compared to the results from this study (in parentheses) healthy volunteers in previous ETB studies showed the following accuracy: 48% (45%) to anger, 50% (52%) to disgust, 52% (50%) to fear, 62% (69%) to happy, 51% (54%) to sad, 56% (59%) to neutral, and 58% (59%) to surprise (Harmer et al. 2003; Harmer et al. 2004). Hence the accuracy levels observed in this study are comparable with those reported in published research.

FERT reaction times were only stable across the last two sessions. This reaction time pattern may relate to the complexity of this task. It has been reported that tasks with complicated instructions are associated with increased practice effects (Powers, 1986). However, using the duel-baseline approach, this task could have utility in repeated-measures experiments if the first two sessions are used as practice sessions.

In the EREC, the number of correctly recalled words increased across the first two sessions, but then stabilised across the final two sessions. As participants did not recall all of the words it is possible that a ceiling effect in memory performance was reached at this point, though there are also other reasons why participants might have stabilised at this level of performance. For the incorrect words, recall was stable after the first session, suggesting quicker learning of incorrect words. Therefore, as with the FERT task, the EREC task appears suitable for repeat testing, after two initial training sessions.

It is possible that using different stimuli for each session would help to reduce practice effects. However, while the use of alternative stimuli reduces practice effects in some studies, the evidence remains inconsistent, and is likely to be task specific (Benedict and Zgaljardic, 1998; Hinton-Bayre and Geggen, 2005). Perhaps more problematic is the issue of whether training on the ETB could bias responding, for example, by training participants to respond according to a response set. Hence, if these tasks are to be used in within-subjects designs, a necessary first step would be to ensure that training will not result in fixed responding. This could be examined by investigating whether responses can be increased or decreased via an experimental manipulation known to produce such effects after several practice trials.

In conclusion, both the ECAT and EMEM are suitable for within-subjects designs, while the FDOT requires a single training session, and the FERT and EREC require two training sessions, if they are to be used repeatedly. Therefore, it may be possible to use the entire battery repeatedly after two initial training sessions. Future work should explore the value of using alternate stimuli across sessions to reduce the effects of practice, and investigate whether repeated testing induces a response set. This would further validate the ETB for use with cross-over designs in experimental medicine models.

4.6. Study 4 Overview and Design

To investigate whether satiety affected performance on the ETB, a between-subjects design with a single factor (condition) and two levels (satiated versus non-fed) was used. Participants were randomly allocated to a condition with 15 participants in each group. Group size was based on previous work in which 12-16 participants per group yielded significant effects on the ETB (Murphy et al. 2008; Harmer et al; 2004; Browning et al. 2007). Based on prior research indicating that mood effects can be reliably detected 60 minutes after food consumption (Smith et al. 1988; Macht and Dettmer, 2006), participants were tested on the ETB 60 minutes after consuming lunch or in a non-fed state.

4.7. Study 4 Methods and Materials

Participants

30 healthy women psychology students (mean age = 21.41 years; mean BMI = 19.97; mean NART = 117) were recruited from the University of Birmingham. Informed consent was obtained from all participants, who were compensated after the study with either course credits or £10 cash. The study was approved by the University of Birmingham Research Ethics Committee and was conducted in accordance with the ethical standards laid down in

the 1964 Declaration of Helsinki. Participants were excluded if they were not: between 18-65 years of age; fluent English speakers as determined by the NART; and of a body mass index (BMI) between 18.5 and 24.9. Participants were also excluded if they had taken part in a previous ETB study, were dyslexic, smokers, currently taking medication, had consumed a large amount of caffeine (> 750mg) or alcohol in the last 24 hours, had food allergies, diabetes, or past or present depression as determined by the SCID. Participants who scored more than 10 on restraint scale of the TFEQ were not recruited. This is because high levels of dietary restraint have been associated with impaired cognitive performance (Green et al. 1994).

Test Meal Setup and Cheese Sandwiches

For lunch, participants were served a platter of cheese sandwiches; sixteen quarters, arranged in two rows of eight quarters each. Each quarter sandwich serving contained 92.3 calories and weighed approximately 31g. Participants were provided with a plate to eat from, and asked to eat as much as they wanted until they felt comfortably full. The platter was weighed before and after serving (along with any remnants left on the participant's plate) to determine total food intake in grams. Participants were also provided with a glass of water.

Procedure

Prior to attending the test session, participants were sent the TFEQ via email to ensure they were eligible for the study. Those who attended the test day completed a consent form, before they were screened with a lifestyle questionnaire, a breakfast questionnaire (to ensure they had not consumed food since 8pm the prior day) the SCID and the NART. Participants also completed an alcohol and caffeine screening questionnaire to assess their intake over the last 24 hours, before completing a set of VAS. VAS items were placed above the centre of a

100mm line, anchored with “not at all” (0mm) and “extremely” (100mm), and included the items: alert; disgusted; drowsy; light-headed; anxious; happy; nauseated; sad; withdrawn; faint; hungry; thirsty; full; and desire to eat.

Participants in the satiated condition were served cheese sandwiches after which they completed another VAS and a sandwich rating questionnaire. This questionnaire assessed liking of the sandwich, whether the meal was a typical size, and whether participants ate beyond comfortable fullness, using VAS scale items. Participants were then asked to wait in a test cubicle for an hour before proceeding to the ETB test; as noted above, mood effects have previously been detected an hour after eating. During this time they completed a VAS after 30 minutes and 60 minutes, the latter immediately prior to ETB testing. Participants were then asked to complete the ETB tasks, followed by a batch of questionnaires, including the PFS, BIS, and BDI. Participants then had their height and weight measured for calculation of BMI, were asked what they thought the aims of the study were, debriefed and thanked for their time. Participants in the non-fed condition completed a similar procedure, but consumed the lunch of cheese sandwiches after completing the ETB tasks.

Data Analysis

General: Between-subjects and mixed analysis of variance (ANOVA) were used to analyse the data. Bonferroni correction was used for all post-hoc t-tests, and violations of sphericity were addressed using the Greenhouse-Geisser correction.

VAS: Items were analysed separately to investigate individual profiles of satiation.

ETB Data: As with Study 3, task specific effects are presented first (e.g. effects of emotion, or valence), followed by main effects of condition, and interactions between condition and emotion or valence.

4.8. Study 4 Results

Participant Characteristics and Subjective State Questionnaires

Using independent t-tests (non-fed versus satiated) no significant differences were observed between groups for age, BMI, NART, BIS, PFS, BDI, TFEQ (cognitive restraint, disinhibition and hunger) and amount eaten (all $p > 0.05$; see Table 4.4). Hence, it appears that the groups were well matched.

Table 4.4 Participant Characteristics and Subjective State Questionnaires (standard error of the mean)

	Condition	
Measure	Non-fed	Satiated
Age	19.7 (0.3)	20.3 (0.5)
Body Mass Index (BMI)	21.5 (0.6)	21.4 (0.5)
National Adult Reading Test (NART)	116.3 (1.1)	117.1 (1.3)
Barratt Impulsivity Scale (BIS)	63.3 (2.0)	68.2 (3.0)
Power of Food Scale (PFS)	38.2 (2.4)	37.4 (3.1)
Beck Depression Inventory (BDI)	5.8 (0.9)	7.8 (1.5)
TFEQ Cognitive Restraint	6.2 (0.8)	6.3 (0.8)
TFEQ Disinhibition	5.3 (0.7)	7.1 (1.0)
TFEQ Hunger	5.4 (1.0)	7.3 (0.9)
Amount Eaten (grams)	193.6 (16.7)	188.5 (15.5)

Three factor eating questionnaire (TFEQ)

Visual Analogue Scales

VAS scores were entered into mixed ANOVAs, with the factor of condition (satiated versus non-fed) and time (pre versus post-manipulation).

Appetite items: Hunger, desire to eat, fullness and thirst showed a main effect of time and a main effect of condition (all $p < 0.001$; see Table 4.5). For hunger, fullness and desire to eat (but not thirst), there were significant interactions between condition and time (all $p < 0.001$;

see Table 4.5). Comparing pre versus post-manipulation ratings separately for each group, rated hunger decreased over time in the satiated ($p < 0.001$; see Table 4.5), but did not change over time in the non-fed group ($p > 0.05$). Desire to eat also decreased over time in the satiated group ($p < 0.001$; see Table 4.5), but increased over time in the non-fed group ($p < 0.05$). Finally, fullness significantly increased over time in the satiated group ($p < 0.001$; see Table 4.5), but did not change in the non-fed group ($p > 0.05$).

Mood items: For alertness and anxiety there were main effects of time whereby alertness and anxiety decreased over time (both $p < 0.05$; see Table 4.5). There was no effect of condition or interaction for either rating. For faint, there was no effect of time or condition (both $p < 0.05$), however, there was a significant interaction between time and condition ($p < 0.01$). Comparing pre versus post-manipulation ratings separately for each group, neither t-test survived significance (both $p < 0.05$), however, the means showed a trend for increased faint ratings in the non-fed group over time, and decreased faint ratings in the satiated group over time. For disgust, drowsiness, light-headed, happiness, nausea, sadness, and withdrawn, there was no effect of time or condition, or a significant interaction (all $p > 0.05$).

Table 4.5 Visual Analogue Scale mean scores split by condition and time (standard error of the mean)

	Non-fed		Satiated	
VAS Item	Pre-Manipulation	Post-Manipulation	Pre-Manipulation	Post-Manipulation
Appetite				
Hunger [a,b,c]	74.5 (3.7)	79.7 (5.6)	81.6 (3.9)	15.3 (5.7)
Desire to Eat [a,b,c]	71.0 (4.0)	81.8 (3.9)	78.9 (4.2)	14.4 (4.0)
Fullness [a,b,c]	14.7 (3.3)	7.2 (4.5)	5.9 (3.4)	64.4 (4.7)
Thirst [a,b]	66.3 (5.1)	52.3 (7.3)	69.4 (5.3)	24.0 (7.5)
Mood				
Alertness [a]	64.5 (5.6)	51.3 (5.6)	59.9 (5.6)	55.8 (5.6)
Disgust	6.6 (1.6)	4.5 (2.1)	2.6 (1.6)	4.6 (2.1)
Anxiety [a]	17.4 (4.8)	10.9 (2.9)	12.0 (4.8)	2.9 (2.9)
Drowsiness	33.4 (7.4)	43.2 (6.5)	36.0 (7.4)	40.5 (6.5)
Lightheaded	25.1 (5.6)	26.7 (5.2)	19.5 (5.6)	8.9 (5.2)
Happiness	60.9 (3.1)	58.7 (3.2)	65.5 (3.1)	67.8 (3.2)
Withdrawn	17.2 (4.7)	18.6 (4.2)	13.3 (4.7)	9.5 (4.2)
Faint [c]	12.1 (5.4)	19.9 (4.7)	19.1 (5.4)	5.5 (4.7)
Sad	11.4 (2.4)	10.5 (3.3)	3.9 (2.4)	6.8 (3.3)
Nausea	10.3 (3.1)	10.1 (4.1)	7.0 (3.1)	5.8 (4.1)

[a] = Main effect of time; [b] = Main effect of condition; [c] = Interaction between time and condition

Emotional Test Battery (ETB) Data

Emotional categorisation task (ECAT) and Emotional recognition memory task (EMEM): There was no effect of valence, condition, nor an interaction between condition and valence (positive versus negative words) for ECAT accuracy (all $p > 0.05$; see Table 4.6). Analysis of ECAT reaction time showed a trend towards a main effect of valence with quicker times for positive versus negative words (F (1 28) = 4.16; $p = 0.05$), but no effect of condition, and no interaction (both $p > 0.05$; see Table 4.6). EMEM accuracy scores showed a main effect of valence with better accuracy for positive versus negative words (F (1 28) = 33.87; $p < 0.001$), but no effect of condition nor a significant interaction (both $p > 0.05$; see Table 4.6). Analysis of reaction time also showed an effect of valence with quicker times for positive versus

negative words (F (1 28) = 54.24; $p < 0.001$), but no effect of condition, nor a significant interaction (both $p > 0.05$; see Table 4.6).

Emotional recall task (EREC): For words correctly recalled, there was a main effect of valence with more positive words recalled versus negative (F (1 28) = 27.80; $p < 0.001$; see Table 4.6), but no effect of condition nor a significant interaction (both $p > 0.05$). For words incorrectly recalled, there was also an effect of valence with more positive words recalled versus negative (F (1 28) = 12.27; $p < 0.01$; see Table 4.6), but no effect of condition nor a significant interaction (both $p > 0.05$).

Facial expression recognition task (FERT): For accuracy, there was a main effect of emotion (F (4 99) = 30.60; $p < 0.001$ – Table 4.7), but no effect of condition, or an interaction between emotion and condition (both $p > 0.05$; see Table 4.8). T-tests on the effect of emotion showed that all facial expressions were categorised significantly less accurately than neutral faces (all $p < 0.01$; see Table 4.8). Analysis of reaction time data also revealed a main effect of emotion (F (4 117) = 19.68; $p < 0.001$ – Table 4.7), but no effect of condition, nor an interaction between emotion and condition (both P > 0.05; see Table 4.8). For the effect of emotion, reaction times to anger, disgust, fear, sadness and surprise expressions were significantly slower than to neutral faces (all $p < 0.01$) (see Table 4.7), while reaction times to happy faces were not significantly different from those to neutral faces ($p > 0.05$; see Table 4.7).

Faces Dot Probe Task (FDOT): For FDOT accuracy and reaction times, there was no main effect of condition (non-fed versus satiated; see Table 4.6), emotion (fear versus happy

faces), masking (masked versus unmasked), or congruency (congruent versus incongruent probe location) and no significant interactions between these factors (all $p > 0.05$).

Table 4.6 Accuracy, Reaction Time, and Number of Correct and Incorrect words recalled for ETB tasks, split by negative and positive stimuli and non-fed and satiated state (standard error of the mean)

		Negative		Positive	
ETB Task	Measure	Non-fed	Satiated	Non-fed	Satiated
Emotional Categorisation (ECAT)	Accuracy	96.7 (1.3)	96.0 (1.3)	96.2 (1.2)	95.3 (1.2)
	Reaction Time	834.7 (41.7)	819.1 (41.7)	785.2 (37.5)	805.0 (37.5)
Emotional Recognition Memory (EMEM)	Accuracy	65.3 (4.4)	62.7 (4.4)	79.8 (2.7)	85.8 (2.7)
	Reaction Time	1081.3 (62.5)	1093.1 (62.5)	915.7 (44.0)	912.1 (44.0)
Emotional Recall (EREC)	Correct Words	5.1 (0.7)	4.7 (0.7)	7.2 (0.7)	7.0 (0.7)
	Incorrect Words	0.6 (0.3)	0.7 (0.3)	1.7 (0.5)	2.7 (0.5)
Faces Dot Probe (FDOT)	Accuracy	95.4 (1.2)	93.2 (1.2)	94.5 (1.5)	92.9 (1.5)
	Reaction Time	630.8 (17.4)	655.5 (17.4)	631.9 (18.1)	656.3 (18.1)

Table 4.7 Accuracy and Reaction Times for FERT Task split by emotion (standard error of the mean)

	Measure	
Emotion	Accuracy	Reaction Times
Anger	46.0 (2.4)***	1504.8 (82.7)***
Disgust	54.8 (1.46)***	1300.2 (55.3)**
Fear	46.7 (2.2)***	1614.5 (67.9)***
Happy	61.8 (1.7)	1179.6 (35.5)
Neutral	78.3 (2.7)***	1124.6 (54.3)
Sad	46.8 (2.9)***	1414.6 (60.0)***
Surprise	58.0 (1.6)***	1387.5 (70.2)**

Significance values are for each emotion versus neutral

**$p < 0.01$; *** $p < 0.001$

Table 4.8 Accuracy and Reaction Times for FERT Task, split by emotions and non-fed and satiated state (standard error of the mean)

	Accuracy		Reaction Times	
Emotion	Non-fed	Satiated	Non-fed	Satiated
Anger	44.0 (3.5)	48.0 (3.5)	1355.6 (112.2)	1654.0 (112.2)
Disgust	54.8 (2.1)	54.7 (2.1)	1291.9 (79.6)	1308.4 (79.6)
Fear	45.8 (3.2)	47.5 (3.2)	1602.3 (97.6)	1626.7 (97.6)
Happy	60.5 (2.4)	63.0 (2.4)	1117.4 (48.3)	1241.9 (48.3)
Neutral	80. (3.8)	76.7 (3.8)	998.3 (70.4)	1250.9 (70.4)
Sad	48.3 (4.1)	45.2 (4.1)	1372.3 (85.6)	1456.9 (85.6)
Surprise	58.8 (2.2)	57.2 (2.2)	1274.9 (96.4)	1500.1 (96.4)

4.9. Study 4 Discussion

Participants who were asked to eat a sandwich lunch until satiated reported a decrease in appetite, compared to participants who were not given lunch. Satiation did not significantly affect questionnaire based measures of mood, nor did it affect performance on the ETB. Hence, the successful induction of satiation did not affect any measures of emotional processing or cognition in this paradigm.

The lack of a satiation-induced effect on mood in the present study might be partly due to the type of food used. The study by Macht and Dettmer (2006) reported that both apple and chocolate consumption elevated mood in healthy women, but the effect of chocolate consumption was greater than the effect of apple consumption. Hence, it is possible that palatable or energy dense foods have greater effects on mood than less palatable or energy dense foods. This suggestion is supported by evidence that foods with a high energy content have greater effects on mood than food with a lower energy content (Macht et al. 2003). Thus, the use of a food that is more palatable or energy dense than bland cheese sandwiches might have elicited effects on emotion, which might then have been detected by the ETB.

Another possible explanation of the lack of effect of lunch consumption on mood might be that any effect was transient and did not persist long enough to be detected by the ETB, which can take up to 90 minutes to complete. Alternatively, satiation may not affect the emotional processing tasks that comprise the ETB. It should be noted that significant effects were observed using the ETB including significant differences between neutral and emotional faces and differences in responding according to valence. Hence, it was possible to detect significant effects on performance in this battery in the laboratory.

The absence of an effect of satiation on cognitive measures from the ETB is not necessarily unexpected. While there is evidence that consumption of food can affect memory (Benton and Parker, 1998), recent work suggests that the type of food consumed is important. For instance, consuming a breakfast of oatmeal enhances cognitive performance compared to a ready-to-go cereal (Mahoney et al. 2005). It is thought that this may be due to oatmeal providing a slow sustained energy release of glucose to the brain, and suggests that the composition of the food consumed is likely to mediate temporal effects on cognition. In addition to evidence suggesting that food consumption has no effect on effect on cognitive performance (Müller et al. 2013), there is also evidence that eating food can directly impair cognitive performance in some tasks (Smith and Miles, 1987). Such effects might be attributable to the well-known "postprandial dip", the effects of which can be worsened by consuming heavier meals (Reyner et al. 2012).

In conclusion, satiation did not affect questionnaire-based measures of mood nor performance on the ETB. Therefore, it seems unlikely that variations in appetitive state due to hunger, eating or drug administration will confound the results of ETB studies. However, further

work with other types of foods such as high calorie cookies given at varying intervals prior to ETB testing would help to confirm this conclusion.

4.10. General Summary

To summarise, after two training sessions the ETB appears to be suitable for use in within-subjects designs, enabling greater control of individual differences which may be particularly important for consistent effects in eating studies. The ETB also appears to be insensitive to the satiety state of participants, which reduces concern about satiety state acting as a potential confound in ETB studies and reinforces the robust nature of the test battery.

CHAPTER 5: Study 5: Natural satiation attenuates blood oxygen level dependent (BOLD) activity in brain regions involved in reward and increases BOLD activity in an inhibitory control centre: a functional magnetic resonance imaging (fMRI) study in healthy volunteers

5.1. Introduction

The use of fMRI has the potential to provide information on brain functional biomarkers of satiation and response to anti-obesity drugs. For instance, recent fMRI work with sibutramine revealed that the drug attenuated BOLD activity in response to viewing of food cues in the hypothalamus and amygdala (Fletcher et al. 2010). Suppression of BOLD activity in the hypothalamus was associated with a greater reduction in ad-libitum food intake and body weight (Fletcher et al. 2010). However, this pattern of activity was different to that observed when comparing BOLD responses in a fasted versus fed state, which suggests that distinct modulation of neural activity might underlie responses to feeding versus drug-induced changes in appetite. Therefore, further work is required to elucidate brain responses to feeding to provide a template against which the actions of anti-obesity drugs can be compared.

Hunger associated with food deprivation increases the incentive value of food, which is reflected in enhanced responses to appetitive stimuli in reward-related brain areas as assessed by fMRI (Goldstone et al. 2009; Führer et al. 2008; LaBar et al. 2001; Haase et al. 2009; Fletcher et al. 2010; Siep et al. 2009). Previous studies have assessed the effects of fasting on brain responses to food-related stimuli (St-Onge et al. 2005; Porubska et al. 2006; Killgore et al.2003), compared the responses of lean and obese participants to eating (Le et al. 2006) or examined differences in brain activation between restrained and unrestrained eaters (Coletta

et al. 2009). Studies that have examined the effects of eating on neural activity have tended to focus on sensory specific responses rather than the effects of satiation per se (Kringelbach et al. 2003; Smeets et al. 2006). To date no study has assessed the effects of a standard meal on BOLD responses and compared this to natural inter-meal hunger levels rather than prolonged fasting. Furthermore, previous studies have only assessed responses to either taste or visual stimuli and did not investigate modality specific effects of satiation on neural responses.

Recently, a role for frontal inhibitory neural circuitry in the control of food intake has been highlighted (see Moran and Westerterp-Plantenga, 2012). For example, dorsolateral prefrontal cortex (dlPFC) activation has been associated with higher levels of self-control over food choices and cognitive restraint of intake (Hare et al. 2009; Hollmann et al. 2012). This raises the possibility that reduced motivation to eat associated with consumption of food (satiation) is mediated in part by enhanced activity in prefrontal brain regions involved in executive control. Interestingly, obese patients with Prader Willi syndrome show hyporeactivity in dlPFC following a meal which has been suggested to be related to the satiety deficit associated with this syndrome (Holsen et al. 2012). Activity in the dlPFC may affect food motivation by modulating reward value signals encoded by the vmPFC (Hare et al. 2009; 2011) suggesting that interactions between dorsolateral and ventromedial PFC may be particularly important in satiation.

In Study 5, an fMRI paradigm was used that was developed to investigate neural responses to both primary and secondary rewarding and aversive appetitive stimuli in the human brain (McCabe et al. 2010). The effects of satiety were assessed by scanning participants after a brief 4 hour fast or following the consumption of a lunch of cheese sandwiches. The use of

this bland food ensured that participants were satiated while minimising sensory specific satiety effects (Kringelbach et al. 2003).

It was predicted that compared to a pre-meal state, consuming a satiating lunch would reduce neural activity in reward and appetitive areas such as ventromedial prefrontal cortex, orbitofrontal cortex, ventral striatum, midbrain, hypothalamus, insula, amygdala, hippocampus and brainstem. It was also predicted that satiation would increase neural responses in the dorsolateral prefrontal cortex (inhibitory control), which would be negatively correlated with activity in the vmPFC, based on recent research showing an inverse relationship between these two areas in a food task (Hare et al. 2009).

5.2. Materials and methods

Participants

16 healthy volunteers (8 men, 8 women; mean age = 21.7 years; mean BMI = 21.1) who met the inclusion criteria were recruited via posters and advertisements on websites. The study was advertised as a chocolate experiment involving fMRI scanning, with a free lunch of cheese sandwiches and £50 compensation. Ethics approval was provided by the Oxford Research Ethics Committee B (National Research Ethics Service) and informed consent was obtained from all participants. Participants were excluded if they had a past or present Axis 1 disorder, as determined by the SCID, or a score over 10 on the BDI. Other exclusion criteria were taking medication, left-handed, smoker, food allergies, diabetic, under 18 or over 60 years of age, outside a BMI range of 18.5 - 24.9 or any contraindications to fMRI scanning such as the presence of a pacemaker.

Design

A within-subjects design was used, with participants taking part in both experimental conditions: pre-meal and satiated. For both conditions, each run on a separate test day, participants were scanned twice, an hour apart. In the satiated condition, participants received lunch between scans, whereas those in the pre-meal condition received lunch after the second scan of the test day, and had a one hour break between scans (Figure 5.1). Participants were tested within a month of screening, and test days were either one or two weeks apart, with scans at the same time and on the same day of the week as the first sessions (+/- half an hour). Order of completing the satiated and pre-meal test days was counterbalanced across participants and gender.

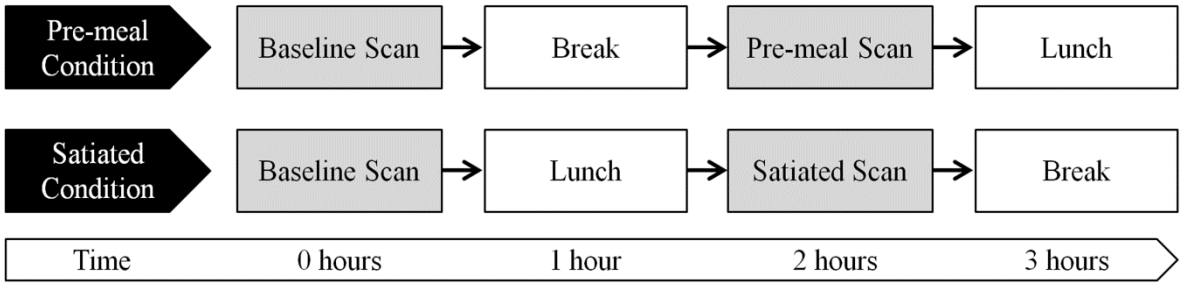

Figure 5.1 Overview of experimental procedure; pre-meal condition and satiated condition with approximate timings.

Scanner Stimuli

Taste stimuli were delivered to participants via three Teflon tubes held between their lips; one tube each for a tasteless rinse control, a chocolate taste and an unpleasant strawberry taste, each of which were liquid at room temperature. The tubes were connected to their own individual reservoir, which was capable of delivering 0.5ml of a given stimulus via a syringe and a one-way syringe activated check valve. Stimulus delivery was provided manually with liquid release over a period of seven seconds in response to computer generated times. The

tasteless rinse control was 0.5 ml of a saliva-like rinse solution (25 X $10^{-}3$ mol/L KCl and 2.5 X $10^{-}3$ mol/L $NaHCO_3$ in distilled H_2O), used between trials, and subtracted from the effects of the other taste stimuli. The aversive strawberry taste stimulus was an unpleasant strawberry drink (Rosemount Pharmaceuticals, Leeds, UK), matched in intensity, sweetness and texture to the chocolate taste stimulus, which was a Belgian Chocolate Milk Drink (Marks & Spencer, UK).

Scanner Task

Based on the experimental paradigm used by McCabe et al. (2010), a total of six conditions (presented nine times) were utilised in the scanner task: chocolate taste; chocolate picture; chocolate taste with chocolate picture; strawberry taste; strawberry picture; strawberry taste with strawberry picture. For trials with oral stimuli, 0.5ml of the appropriate liquid was delivered to the participant's mouth while they were inside the scanner. Simultaneously, a visual stimulus was presented either of chocolate, mouldy strawberries or a gray control image for seven seconds. Participants were instructed to move their tongue once, as soon as the liquid was administered to disperse it throughout the mouth, and then to remain still until a green cross was shown, signalling to the participant to swallow the liquid.

After a 2 second delay, participants rated each of the stimuli on that trial for: "pleasantness" (anchored at +2 for very pleasant, 0 for neutral, and -2 for very unpleasant); "intensity" (0 to 4, with 4 being most intense); and for "wanting" (using the same scale as pleasantness). They did this by moving a bar vertically along a scale, using a button box. The ratings were followed by presentation of the visual gray control stimulus and the tasteless control solution, in the same manner as the test stimuli. The tasteless control solution was always administered whilst displaying the gray control stimulus. On trials where only the chocolate or strawberry

image was presented, the rinse was not used but the gray control image was shown to allow a contrast. This sequence was followed by a two second delay for swallowing, followed by a one second gap between trials. This procedure was repeated for each of the six conditions, 9 times.

Lunch

The lunch consisted of a glass of water and an ad-libitum meal of cheddar cheese sandwiches on oatmeal bread. A single sandwich weighed approximately 120g (358 calories) and participants were able to eat a maximum of eight sandwiches (960g). Four sandwiches were initially provided to each participant, and they were given free access to additional sandwiches if they wished to consume more.

Procedure

Screening Day: Participants were screened with the Medical Screening Sheet, SCID and BDI, to determine eligibility. They were also given the: Fawcett-Clarke Pleasure Scale to examine their capacity for pleasure (FCPS – Fawcett et al., 1983), Snaith-Hamilton Pleasure Scale as an indicator of anhedonia (SHAPS – Snaith et al., 1995), Eating Attitudes Test questionnaire as a measure of disordered eating (EAT – Garner et al., 1982), and the TFEQ. Participants also completed an fMRI screening sheet to ensure they were eligible to take part. Their height and weight was taken to allow calculation of BMI and they completed a trial run of the scanner task to ensure they liked and disliked the chocolate and strawberry liquids, respectively. Participants also ate a sample of the cheese sandwiches to ensure they would be willing to eat the same sandwiches on the test days. A Sandwich Rating Questionnaire (SRQ) was used to assess liking of the cheese sandwich; the questionnaire used 100mm VAS and asked participants to rate the sandwich on how enjoyable it was and whether they would

choose to eat it. A Chocolate Questionnaire (CQ) was also used to assess chocolate craving, liking, frequency of consumption and amount consumed.

Testing Day: Participants were asked not to eat chocolate for 24 hours before the scan and this was checked verbally on the morning of the test. They were also asked not to eat food for at least 2 hours prior to scanning to ensure a pre-meal state and this was checked by having participants complete a breakfast questionnaire detailing when they last ate. Scanning took place either between 11.30am – 2.30pm or 12.30pm – 3.30pm. On arrival, participants filled out a breakfast form, the STAI and VAS, before completing the first scan of the day. Each VAS questionnaire used 100mm scales, and contained mood and appetite related items: alertness; disgust; drowsiness; anxiety; happiness; nausea; sadness; withdrawn; faint; hungry; full; desire to eat; thirst. After this, participants completed another set of appetite VAS. Participants in the satiated condition were invited to consume their lunch and asked to eat to the nearest half sandwich to facilitate estimating the amount of food eaten, either at 12.30pm or 1.30pm, depending on starting time. After they had finished eating, participants were asked to fill in a sandwich rating form and appetite VAS, before completing the second scan. After the second scan, participants completed a final set of appetite and mood VAS and a STAI. Participants in the pre-meal condition, completed appetite VAS after the first scan and were then given a one hour break outside the scanner instead of eating lunch. During this time, participants were allowed to read a book or a newspaper in an adjacent room. This break was followed by an appetite VAS and the second scan. Subsequently, participants were given an appetite VAS and lunch as above, with the same sandwich rating, before completing a final set of mood and appetite VAS, along with a STAI. Participants then returned for a second scanning day, and underwent the entire procedure again, for the condition they did not complete the first time.

fMRI Scan

An event-related interleaved design utilised the six scanner task stimuli described above in random permuted sequence. A 3.0 T Magnetom Verio (Siemens) whole body scanner was used at the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB), acquiring T2*weighted echo planar imaging (EPI) slices every 2 seconds (TR = 2). 36 Axial slices (in-plane resolution of 3 x 3 x 3 mm - no gap) were acquired, the matrix size was 64 x 64 and field of view was 192 x 192 mm. Acquisition took place during the task, resulting in a total of 976 volumes, the first four being dummy scans. A whole brain T2*-weighted EPI volume of these dimensions and an anatomic T1 volume with axial plane slice thickness of 1 mm and in-plane resolution of 1.0 x 1.0 x 1.0 mm were also obtained.

fMRI Analysis

FMRIB software library (FSL; FMRIB, Oxford, www.fmrib.ox.ac.uk/fsl) was used for pre-processing and statistical analyses of the data. For pre-processing, the following were used: high pass filter cut off of 60s; motion correction using FMRIB's Linear Image Registration Tool (MCFLIRT); interleaved slice timing correction; spatial smoothing with a 6mm full-width-half-maximum kernel; high pass temporal filtering and film pre-whitening. Functional data were registered to their corresponding structural images and transformed to Montreal Neurological Institute (MNI) space using a reference brain (12 DOF linear transformation). Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) was also used to remove artefacts; mean percentage of components removed = 4.98%; SE = 0.27%.

Following this, the MELODIC filtered scans were entered into a first level analysis, to produce contrasts of the 6 experimental stimuli from the task, minus the corresponding

control stimuli (control rinse, control gray image and combination of both). Motion correction parameters from MELODIC for each of the 6 experimental stimuli were entered as regressors of no interest and orthogonalised to the total of 9 experimental and control stimuli. To account for baseline, a second level analysis (Fixed Effects) was run on these first level outputs for each participant to subtract baseline scans from the post manipulation scans (i.e. pre-meal minus baseline and satiated minus baseline). Subsequently, all second level outputs were entered into a third level mixed effects (FMRIB's Local Analysis of Mixed Effects 1+2; FLAME 1+2) group analysis, producing f-tests for the following main effects and interactions: condition; stimuli; modality; condition x stimuli; condition x modality; stimuli x modality and condition x stimuli x modality. Using a backwards elimination process, non-significant variables were removed one at a time (interactions first, followed by main effects) until only variables that were significant remained.

Group Z statistic images were subsequently corrected for multiple comparisons by means of family wise error (FWE) correction to control for false positives using the AlphaSim program, part of the AFNI toolkit (Cox, 1996). To control the FWE rate, the program takes the particular voxel-wise threshold, voxel dimensions and spatial smoothing kernel size used in the fMRI analysis and, by means of a Monte Carlo simulation, computes the probability of a cluster of specific size arising by chance. Based on our data, with a voxel-wise threshold of $p < 0.001$ ($Z > 3.1$) only clusters with more than 24 contiguous voxels were significant with a FWE corrected $p < 0.05$. As the hypothalamus is difficult to image accurately due to its proximity to sinuses and susceptibility to artefacts (Ojemann et al. 1997) and it is a particularly small region yielding small cluster sizes in other appetite-fMRI studies (Haase et al. 2009), a more conservative voxel-wise threshold ($p < 0.0005$; $Z > 3.3$) producing a smaller FWE cluster threshold (19 or more contiguous voxels; $p < 0.05$) was used to balance the risk

of Type 1 versus Type 2 errors. Effects of stimuli and modality were not reported unless they interacted with satiation. For clarity, local maxima are reported in the tables of activation results. Brain regions were anatomically identified using FSL's integrated Harvard-Oxford Cortical and Subcortical Atlases. For regions not covered by these the Duvernoy Atlas (Duvernoy, 1999) was used. To display brain regions of interest, group Z statistic images were masked using a mask of the brain region (derived from cluster parameters) and then entered into MRIcro (Rorden & Brett, 2000) to visualise BOLD signal % change.

Behavioural Data Analysis

Appetite VAS taken pre- and post-lunch and break for those who were satiated and pre-meal respectively, were averaged over time for hunger, desire to eat and fullness. All three items were correlated significantly (Pearson's correlations, all $p < 0.01$) and so they were averaged into the composite measure 'appetite' for analysis. Scanner task ratings were averaged across the scanning session and, along with region of interest data, were analysed by condition (pre-meal versus satiated), stimuli (chocolate versus strawberry) and modality (taste, picture, and taste and picture). Only main effects of condition and interactions with condition were followed up with planned comparisons. All t-tests used Bonferroni correction and violations of sphericity were addressed using the Greenhouse-Geisser correction.

5.3. Results

Participants

All participant scores for the TFEQ, STAI, FCPS, SHAPS, BDI and EAT questionnaires were within the normal range, and liking and craving ratings of chocolate were sufficient to ensure chocolate would be a robust reward stimuli (see Table 5.1). In addition, the food intake of participants in the pre-meal condition, who ate 336.9g (SE = 40.5g; lunch =1004

calories), did not differ from that of participants in the satiated condition, who ate 398.1g (SE = 41.8g; lunch = 1186 calories) ($p > 0.05$).

Table 5.1: Participant characteristics and baseline means (standard error of the mean)

Measure	Mean (SE)
TFEQ Restraint Scale	3.8 (0.8)
TFEQ Disinhibition Scale	4.6 (0.6)
TFEQ Hunger Scale	3.9 (0.8)
STAI State	30.1 (1.7)
STAI Trait	30.4 (1.7)
FCPS	131.5 (3.7)
SHAPS	22.3 (1.0)
BDI	0.8 (0.4)
EAT	2.4 (0.5)
Chocolate Liking	8.4 (0.3)
Chocolate Craving	6.1 (0.4)
Pre-meal Condition Intake (grams)	336.9 (40.5)
Satiated Condition Intake (grams)	398.1 (41.8)

TFEQ, Three Factor Eating Questionnaire; STAI, State and Trait Anxiety Inventory; FCPS, Fawcett Clarke Pleasure Scale; SHAPS, Snaith-Hamilton Pleasure Scale; BDI, Beck Depression Inventory; EAT, Eating Attitudes Test questionnaire.

Appetite VAS

There was a main effect of condition (F (1 15) = 95.91; $p < 0.001$), and of time (F (1 15) = 9.63; $p < 0.01$) and an interaction between condition and time (F (1 15) = 59.13; $p < 0.001$.) There were no significant differences between baseline appetite for the satiated and pre-meal conditions (52.9mm versus 55.9mm; t (15) = -0.55, $p > 0.05$), but appetite was significantly higher in the pre-meal than the satiated condition (73.3mm versus 11.1mm; t (15) = 13.37, $p < 0.001$).

Scanner Task Subjective Ratings

Pleasantness: Mean ratings from the scan during which participants were in the pre-meal or satiated state were analysed by condition, stimuli (chocolate versus strawberry) and modality (taste, picture, and taste and picture). There was a main effect of condition (F (1 15) = 8.59; $p < 0.05$), stimulus (F (1 15) = 270.36; $p < 0.001$) as well as interactions between condition and stimuli (F (1 15) = 11.34; $p < 0.01$) and between stimulus and modality (F (1 20) = 5.66; $p < 0.05$). Chocolate stimuli were rated significantly less pleasant when satiated than in the pre-meal state (t (15) = -3.79, $p < 0.01$, see Figure 5.2). In contrast, there were no significant differences for ratings of strawberry stimuli between the pre-meal and satiated conditions (t (15) = 1.63, $p > 0.05$).

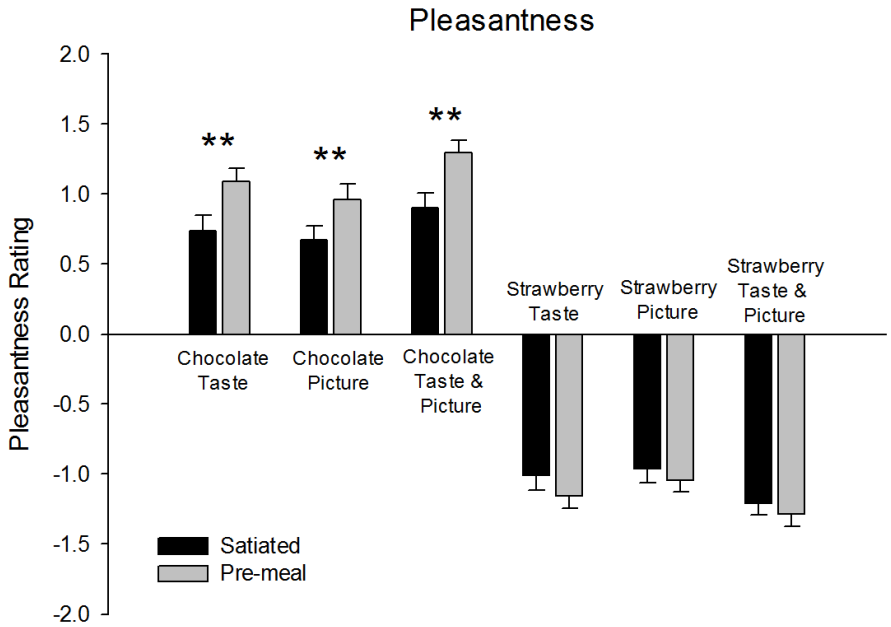

Figure 5.2. Mean pleasantness ratings for chocolate and strawberry stimuli, split by modality and condition (with standard error bars). **$p < 0.01$

Wanting: There was a main effect of condition (F (1 15) = 25.97; $p < 0.001$), stimulus (F (1 15) = 268.13; $p < 0.001$) and interactions between condition and stimulus (F (1 15) = 26.52; $p < 0.001$) and stimulus and modality (F (2 30) = 6.96; $p < 0.01$). Chocolate stimuli were rated significantly less wanted in the satiated condition compared to the pre-meal condition (0.55 versus 1.22; t (15) = -6.15, $p < 0.001$, see Figure 5.3). There were no significant differences between ratings in the pre-meal and satiated conditions for strawberry stimuli (t (15) = 0.39, $p > 0.05$).

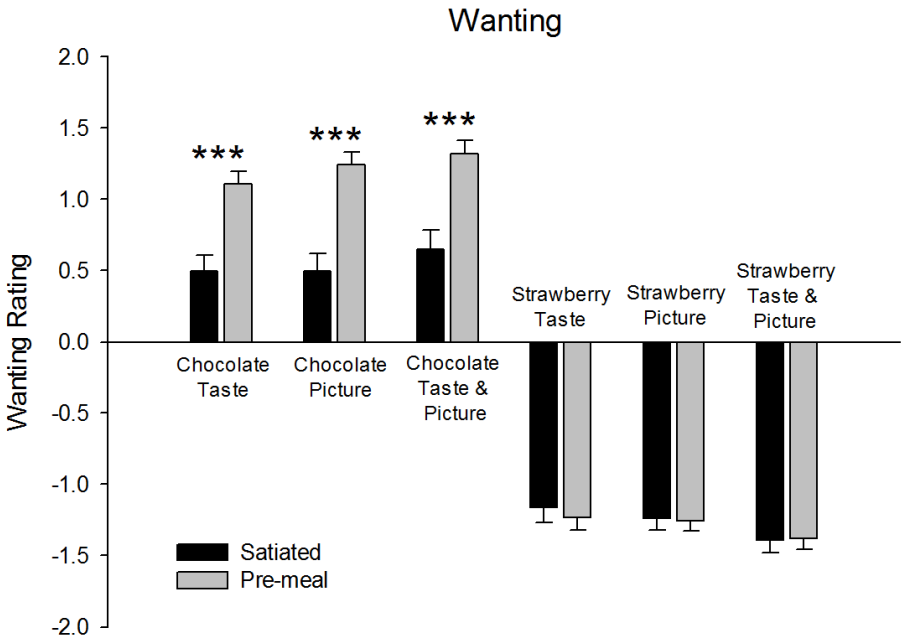

Figure 5.3. Mean intensity ratings for chocolate and strawberry stimuli, split by modality and condition (with standard error bars). ***$p < 0.001$

Intensity: There was a main effect of stimulus (F (1 15) = 15.07; $p < 0.01$ – Figure 5.4) with the strawberry stimuli rated more intense than the chocolate stimuli and of modality (F (1 15) = 20.14; $p < 0.001$). There was also a trend towards an effect of condition (F (1 15) = 3.45; $p = 0.08$), with higher intensity ratings when in the pre-meal state compared to satiated.

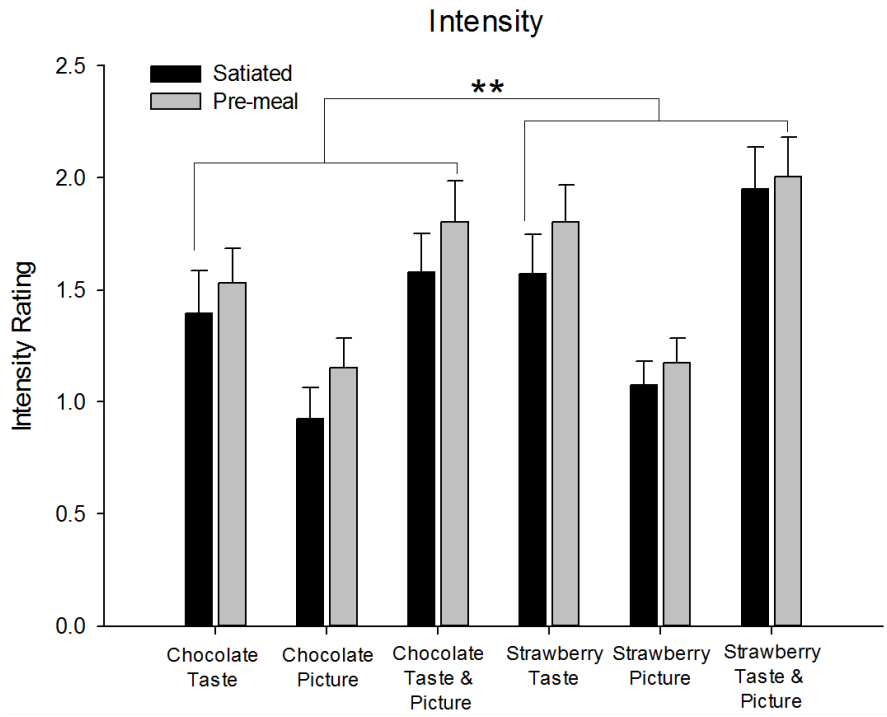

Figure 5.4. Mean intensity ratings for chocolate and strawberry stimuli, split by modality and condition (with standard error bars). $^{**}p < 0.01$

fMRI Whole Brain Analysis

Contrast of parameter estimates (COPEs) for each of the six experimental stimuli (chocolate taste, chocolate picture, chocolate taste and picture, strawberry taste, strawberry picture, and strawberry taste and picture) had the corresponding control stimuli subtracted at the first level (control rinse, control gray image and combination of both). At the second level, baseline scans at the start of the day were subtracted from scans taken during the pre-meal (e.g. pre-meal – baseline scan) and satiated state (e.g. satiated state – baseline scan). These contrasts were then entered into a higher level analysis with the following factors: condition (pre-meal versus satiated); stimulus (chocolate versus strawberry) and modality (taste versus picture versus taste and picture). There was a main effect of condition and a main effect of modality; however, there was no main effect of stimulus and nor any significant interactions between

any of the factors. Local maxima for the main effect of condition are reported for key appetitive/reward areas (see Table 5.2). Additional areas showing an effect of condition are reported in Table 5.3 for reference. Satiation attenuated BOLD activity in the ventromedial prefrontal cortex (vmPFC), orbitofrontal cortex, nucleus accumbens (ventral striatum), hypothalamus and insula (see Figures 5.5 and 5.6). Activity was not attenuated in the amygdala or hippocampus at this statistical threshold. Stimulus-evoked BOLD activity was enhanced in the satiated state; bilaterally in the dorsolateral prefrontal cortex (dlPFC) and the insula (see Figures 5.7 and 5.8). To investigate the relationship between the vmPFC (reward) and dlPFC (inhibitory control), percentage signal change values for the local maxima were extracted and correlated using SPSS for the left vmPFC and dlPFC while satiated. A Pearson's correlation coefficient, revealed a significant negative correlation ($r = -0.580$, $n = 16$, $p < 0.05$, see Figure 5.9).

As additional checks, there were no significant differences in BOLD response between men and women, and BOLD response to the stimuli did not change over time (baseline scan day 1 compared to baseline scan day 2).

Table 5.2. Local maxima of key appetitive and reward areas showing main effect of condition (satiation versus pre-meal state) averaged over stimuli and modality.

Brain Region (Hemisphere)	Montreal Neurological Institute (MNI) Coordinates			Estimated Brodmann Area	Z Score
	X	Y	Z		
Reduced activation when satiated (versus pre-meal state)					
Ventrolateral PFC (R)	50	42	-12	47	4.0
Ventromedial PFC (L)	-12	52	-22	11	4.0
Nucleus Accumbens (L)	-8	4	-12	--	3.5
Orbitofrontal Cortex (R)	46	32	-12	47	3.5
Anterior Medial PFC (R)	8	68	12	10	3.4
Hypothalamus (L) *	-8	-6	-18	--	3.4
Insula (L)	-42	2	-12	48	3.1
Ventrolateral PFC (L)	-44	48	-18	47	3.1
Increased activation when satiated (versus pre-meal state)					
Dorsolateral PFC (R)	46	46	16	46	4.8
Anterior Lateral PFC (L)	-44	56	0	46	4.3
Parahippocampal G (L)	-16	-30	-10	30	4.3
Dorsolateral PFC (L)	-42	50	16	46	4.1
Insula (R)	38	-6	14	48	4.0
Parahippocampal G (R)	20	-18	-24	35	3.7
Anterior Medial PFC	0	56	0	--	3.4

FWE cluster corrected (voxel $p < 0.001$; cluster > 24 contiguous voxels – $p < 0.05$).
Prefrontal Cortex (PFC); Gyrus (G); Left side (L); Right side (R).
*Hypothalamus FWE cluster corrected (voxel $p < 0.0001$; cluster > 15 contiguous voxels - $p < 0.05$).

Table 5.3. Local maxima of other brain areas showing main effect of condition (satiation versus pre-meal state) averaged over stimuli and modality.

Brain Region (Hemisphere)	Montreal Neurological Institute (MNI) Coordinates			Estimated Brodmann Area	Z Score
	X	Y	Z		
Reduced activation when satiated (versus pre-meal state)					
Frontal G (R)	16	18	62	8	4.5
Temporal Pole (L)	-36	8	-22	38	3.3
Increased activation when satiated (versus pre-meal state)					
Superior Parietal Cortex (R)	30	-44	68	1	5.6
Temporal G (R)	48	-50	-12	20	5.5
Occipital Cortex (R)	50	-74	-2	19	5.5
Occipital Cortex (L)	-40	-80	-8	19	5.4
Postcentral G (L)	-66	-14	22	43	5.2
Superior Parietal Cortex (L)	-38	-42	54	40	5.2
Heschl's G (R)	52	-22	8	48	5.0
Supramarginal G (L)	-66	-28	32	2	4.9
Lingual G (R)	4	-66	0	18	4.7
Precuneous Cortex (L)	-2	-58	32	--	4.6
Occipital Fusiform G (L)	-24	-66	-12	19	4.4
Temporal G (L)	-50	-48	-24	20	4.4
Precuneous Cortex (R)	24	-52	6	19	4.3
Supramarginal G (R)	62	-32	44	40	4.1
Lingual G (L)	-10	-50	0	18	4.1
Postcentral G (R)	52	-16	40	3	4.1
Precentral G (R)	32	-6	70	6	4.1
Thalamus (R)	14	-24	12	--	4.0
Occipital Fusiform G (R)	40	-66	-12	19	3.9
Frontal G (R)	36	32	44	9	3.8
Precentral G (L)	-42	-14	62	4	3.5

FWE cluster corrected (voxel $p < 0.001$; cluster > 24 contiguous voxels – $p < 0.05$). Gyrus (G); Left side (L); Right side (R).

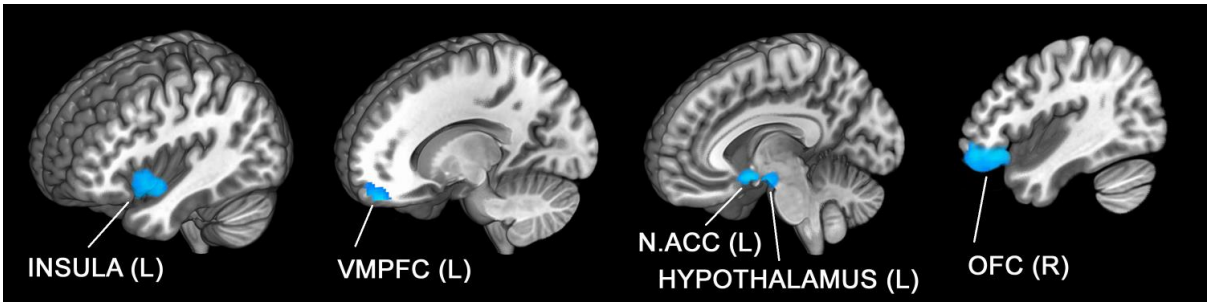

Figure 5.5 Brain regions in blue showed a reduced BOLD signal when satiated compared to pre-meal. Insula; Ventromedial prefrontal cortex (VMPFC); Nucleus Accumbens (N.ACC); Hypothalamus (HYPO); Orbitofrontal Cortex (OFC).

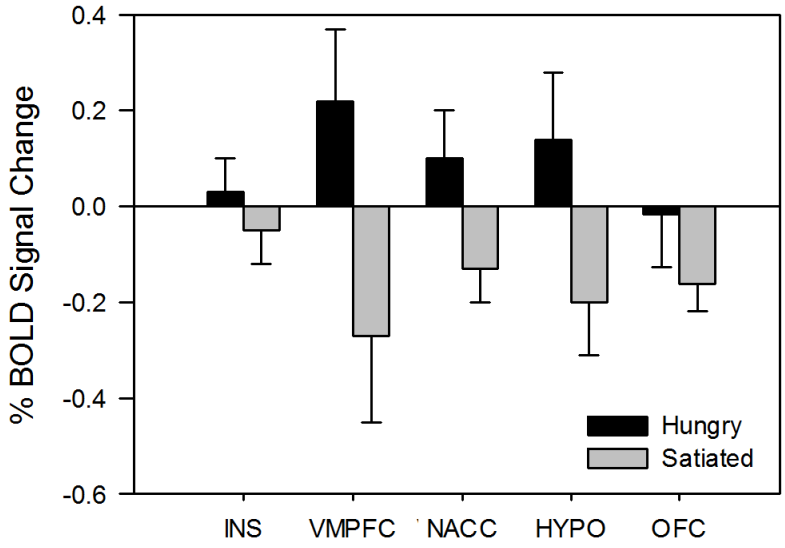

Figure 5.6 Percentage change in areas showing a reduced BOLD signal when satiated (compared to pre-meal). Insula (INS); Ventromedial prefrontal cortex (VMPFC); Nucleus Accumbens (NACC); Hypothalamus (HYPO); Orbitofrontal Cortex (OFC).

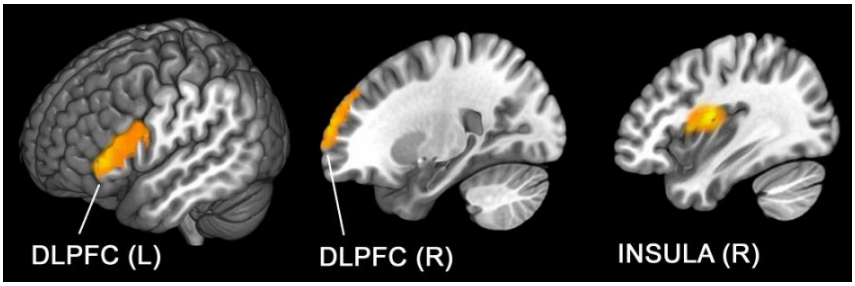

Figure 5.7 Areas in yellow **(**Dorsolateral Prefrontal Cortex (DLPFC) and Insula) showed enhanced stimulus-evoked BOLD activity when satiated (versus pre-meal).

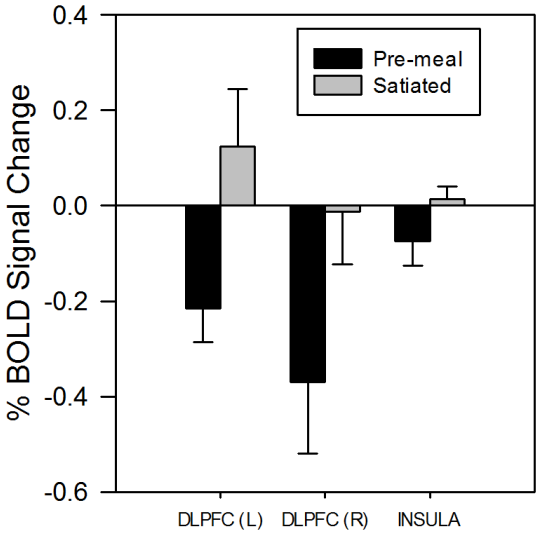

Figure 5.8 Percentage signal change in left and right dorsolateral prefrontal cortex (DLPFC) and insula when satiated.

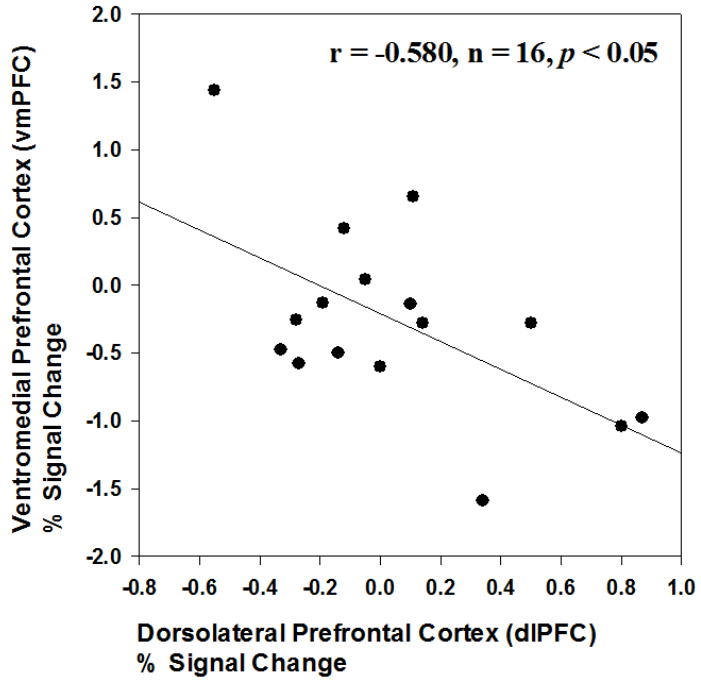

Figure 5.9 Negative correlation between percentage change values in BOLD signal in left dorsolateral prefrontal cortex (DLPFC) and ventromedial prefrontal cortex (vmPFC) while satiated.

5.4. Discussion

A novel fMRI paradigm was used to assess the effects of satiation on brain responses to aversive and rewarding food-related tastes and pictures. Satiation of healthy volunteers attenuated stimulus-evoked BOLD activity across key reward areas and enhanced stimulus-evoked BOLD activity in areas associated with inhibitory control. Activity in the vmPFC was negatively correlated with activity in the dlPFC. Importantly, this pattern of results was observed during normal eating patterns and therefore the profile of activity is likely to reflect brain patterns associated with natural satiation; this can be used as a benchmark in the experimental medicine model for future fMRI studies examining the neural profiles of satiation enhancing drugs.

Multiple reward areas were attenuated by satiation and the model appears to be sensitive to changes across the striatal-cortical pathway. Attenuation of the nucleus accumbens, ventromedial prefrontal cortex and orbitofrontal cortex is consistent with satiety induced decreases in reward (Small et al. 2001; Fletcher et al.2010; Kringelbach et al. 2003), reinforcing the involvement of these reward areas in energy balance. This is a significant finding, as previous paradigms typically report more limited profiles of attenuation: e.g. orbitofrontal cortex (Kringelbach et al. 2003) or ventral striatum (Fletcher et al.2010). Increased stimulus-evoked BOLD activation in these areas has been reported in obese versus healthy individuals (Stoeckel et al. 2008; DelParigi et al. 2005). Therefore, the results of the present study reinforce the suggestion that reduced appetitive reward value is likely to be part of usual satiation.

We also observed that satiation increased stimulus-evoked dlPFC BOLD activity after a meal, and this was negatively correlated with vmPFC activity. Although we did not measure inhibitory control directly in this study, previous work has reported that increased dlPFC activity reflects increased cognitive/inhibitory control (MacDonald et al. 2000). Hare and colleagues (Hare et al. 2011) have reported that the dlPFC modulates vmPFC reward responses to food, suggesting that enhancement of inhibitory control is a mechanism of limiting further food intake by blunting reward. Interestingly, it has been reported that the strength of the connection between the dlPFC and vmPFC is associated with dietary success in obesity (Weygandt et al. 2013). Furthermore, such a mechanism may underlie overeating in obesity. Obesity is associated with reduced behavioural inhibition and reduced neural activity in inhibitory control areas when satiated (Le et al. 2006; Appelhans et al. 2011; Martens et al. 2013). This suggests that restoration of the dlPFC response to food stimuli may be a potential target for obesity treatment. Indeed non-invasive stimulation of this region reduces craving for highly palatable foods (Jansen et al. 2013). Recent studies with the

selective noradrenaline reuptake inhibitor atomoxetine have reported enhanced dlPFC activation in children with attention deficit hyperactivity disorder (ADHD) (Cubillo et al. 2013) and weight-loss in obese individuals (Gadde et al. 2006). The behavioural mechanism of this effect has not been delineated but it could be concluded, based on the results of the present study, that the drug may enhance satiation by increasing dlPFC control over the reward response in the vmPFC. Further studies with atomoxetine in the current model are required to test this hypothesis.

For the first time, a satiation-induced decrease in hypothalamic activity was identified in response to food taste and food pictures that was not confounded by sensory specific satiety or overeating (Smeets et al. 2006; Cornier et al. 2009). It has been reported previously that administration of the anorectic drug sibutramine reduces hypothalamic responses to pictures of high calorie foods (Fletcher et al. 2010). However, these authors did not observe a reduction in hypothalamic activity after participants consumed a standard breakfast following an overnight fast. It is possible that the effects of satiety on hypothalamic responding are dependent upon the baseline level of hunger and whether participants are allowed to eat to fullness.

Insula activity showed a more complex pattern of response, with increased superior activity and decreased inferior activity, when satiated. Increased activation of the superior insula may represent an aversion to further food ingestion associated with satiation (Krolak-Salmon et al. 2003; Wicker et al. 2003) while decreased inferior insula activity may relate to bodily sensations of fullness (Wang et al. 2008).

Participants showed a decrease in wanting and pleasantness of the rewarding chocolate stimuli but not the aversive strawberry stimuli when satiated. This finding is consistent with

the idea that eating-related declines in rated food pleasantness are more pronounced for hedonically positive than aversive stimuli (Scherr et al. 1982). The hedonic valence of aversive tastes may be more resistant to change because avoidance of tastes such as sour and bitter, which normally signal the presence of toxins in food, has adaptive value (Capaldi et al. 1997). Although this interaction was not observed for the BOLD signal, there are several reasons why this might be the case. For instance, Berridge (1996) argues that subjective ratings of reward can be distorted by conscious awareness of stimuli (i.e. cognitive processes). Hence, it is possible that the subjective ratings are not a true reflection of underlying brain activity related to hedonic responses. Alternatively, it might be that the brain regions in which satiation interacts with stimulus type (chocolate versus strawberry) are difficult to image. For instance, the brainstem in decerebrate rats is responsive to aversive and rewarding stimuli (Grill et al. 2002), but is very difficult to image accurately in humans (Brooks et al. 2013b).

Intensity ratings were also recorded, and the lack of significant reductions when satiated supports evidence that the intensity of the stimulus, and potentially the neural circuitry underlying it, are dissociable from rated pleasantness and wanting (Small et al. 2003).

In conclusion, the effect of natural satiation on brain responses to food stimuli has been profiled for the first time using a novel fMRI paradigm. Satiation was associated with attenuated stimulus-evoked BOLD activity in reward-related areas and enhanced BOLD activity in an area associated with inhibitory control. The model provides behavioural and neuronal markers of satiety that may be compared with the effects of putative satiety enhancing agents to facilitate the development of novel treatments for appetite control and obesity.

CHAPTER 6: Study 6: Effect of acute administration of meta-Chlorophenylpiperazine (mCPP) on appetite and fMRI BOLD signals in healthy women.

6.1. Introduction

The results from Study 1 demonstrated that it was possible to detect the effect of a single dose of mCPP on eating behaviour using a UEM. The results from Study 5 showed that it was possible to detect the effects of satiation on brain activity, using fMRI. The aim of the present study was to extend this work by examining the effects of a single dose of mCPP on brain responses to appetitive stimuli using fMRI, and to extend the experimental medicine model to include the capacity to detect selective neural and behavioural responses to foods which vary in energy density and palatability.

fMRI provides a useful method for exploring the neural basis of food preferences, and their modulation by various appetitive states. Thus, high calorie compared to low calorie food images induce greater activity in reward and appetitive brain regions, including: frontal cortex; insula; striatum; midbrain; hypothalamus; amygdala; hippocampus; and anterior cingulate cortex (Frank et al. 2010; Fletcher et al. 2010; Haase et al. 2009; van der Laan et al. 2011). Further, this increased response to high calorie foods is modulated by appetitive state, with fasting/hunger selectively increasing activation to high calorie foods compared to low calorie foods in ventral striatum, amygdala, insula, orbitofrontal cortex, caudate, putamen and cingulate cortex (Goldstone 2009; Siep 2009).

It is possible that drugs affecting appetitive state also have a selective effect on brain responses to food images that is dependent on the caloric value of the food. This idea is supported by recent work showing that administration of the satiety enhancing anti-obesity

drug sibutramine selectively attenuated activity in response to high but not low calorie foods in the hypothalamus and amygdala (Fletcher et al. 2010). Cambridge and colleagues (2013) also reported that the anti-obesity/binge eating novel μ-opioid receptor antagonist GSK1521498 decreased activation in the pallidum/putamen to high calorie, but not low calorie, food images. Other anti-obesity drugs may also have selective effects on high versus low calorie foods; such an effect might be helpful in reducing the consumption of highly palatable energy dense foods.

Brain responses to food images are associated with food intake (Fletcher et al. 2010), which suggests that selective neural effects detected by brain imaging might be reflected in eating behaviour. This is consistent with the recent finding that sibutramine selectively reduced the binge eating of lard by rats (Popik et al. 2011). Using a UEM it is possible to detect drug induced effects on eating behaviour in both obese and lean individuals (Halford et al. 2010; Study 1). Therefore, by adapting this method to include a food which is energy dense and highly palatable and a staple food which is less energy dense and palatable, it may be possible to investigate selective drug effects on eating microstructure for these foods.

Recent work examining the effect of mCPP on the BOLD response in humans has reported that intravenous administration of mCPP increases activity in the: choroid plexus, substantia nigra, hypothalamus, caudate, pallidum, amygdala, pyriform cortex and anterior cingulate gyrus (Anderson et al. 2002b; McKie et al. 2011). 5-HT_{2C} receptors have been identified in the human brain in the choroid plexus, substantia nigra, hypothalamus, basal ganglia, amygdala, hippocampus and prefrontal cortex (Marazziti et al. 1999; Pazos, 1987). Hence, there is clear overlap between the brain distribution of 5-HT_{2C} receptors, and the effect of

mCPP on the fMRI BOLD signal. Thus mCPP is an appropriate compound to probe the effects of 5-HT_{2C} receptor stimulation on neural activity, using fMRI.

The present study examined the effects of a single dose of mCPP versus placebo on BOLD fMRI responses to images of low and high calorie foods in healthy women volunteers. The effects of mCPP on ad-libitum consumption of a tomato based pasta meal, followed by ad-libitum intake of a chocolate chip cookie snack (high energy density/palatability) were also examined. It was hypothesised that mCPP would reduce the BOLD response to food images in key reward and appetitive areas (frontal cortex, insula, hypothalamus, amygdala, hippocampus, nucleus accumbens, caudate, putamen, cingulate cortex and brainstem), and that these effects would be more pronounced or selective for high calorie foods, than low calorie foods. It was also hypothesised that there would be a parallel effect on eating behaviour measured by the UEM, whereby mCPP would decrease eating behaviour measures for pasta and cookies, but have a greater or selective effect on cookie intake.

In addition, behavioural responses to anti-obesity drugs often vary with some individuals responding and showing decreases in food intake while others show no effect (Barkeling et al. 2003). Therefore, as a secondary aim, the study aimed to differentiate drug responders from non-responders and to investigate differences in behavioural and neural responses to food stimuli that might account for any differential responses.

6.2 Methods and Materials

Participants

24 healthy women volunteers (mean age 22.71 (SE: 1.19); mean BMI 21.79 (SE: 0.32); mean cognitive restraint score 5.71 (SE: 0.49) were recruited from the University of Birmingham.

Posters advertised the study as an "Appetite & fMRI study", and participants were compensated with £100 upon completion. Psychology students were also able to complete the study for credits as part of their course requirement. Informed consent was obtained for all participants and ethical approval was provided by South Birmingham Research Ethics Committee (National Research Ethics Service). The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Participants were screened to exclude the following: under 18 or over 65 years old; BMI under 18.5 or over 24.9; English not first language as determined by the NART; taking psychotropic medication; past or current Axis 1 disorder as determined by the SCID; pregnant or breastfeeding; smoker; dyslexic; food allergies; diabetic; cognitive restraint score higher than 10 as measured by the TFEQ. Participants were also excluded if they had previously taken part in an mCPP study, were left-handed, or had any contraindications to fMRI scanning such as pacemakers assessed via an fMRI Screening Form (FSF). Women were asked to participate in test days that fell outside their premenstrual week.

Design

A repeated-measures, double-blind, placebo controlled design was used. There was one factor: drug condition was a within-subjects factor with two levels consisting of mCPP 30mg and placebo administration on separate test days. mCPP was supplied by the Guy's and St Thomas' NHS Foundation Trust Pharmacy Manufacturing Unit (GSTFT). The 30mg dose was selected on the basis of the results of Study 1. To maintain blinding, mCPP and placebo were prepared in identical capsules. Awareness of the UEM was investigated as a secondary aim in this study: the first 12 participants were not made aware of its presence (non-aware) while the second 12 participants were made explicitly aware that it was being used (aware). For the analysis of responders versus non-responders, individuals were identified as

responders if they showed more than a 10% decrease in food intake for the mCPP versus placebo condition. Non-responders were classified as those showing less than a 10% decrease in intake for mCPP versus placebo.

Universal Eating Monitor (UEM)

Food was served on a UEM (see Study 1 for details).

Pasta Lunch: Dishes filled with 220g of pasta were placed on the placemat. Each time the participant ate 50g of the pasta, the SIPM software (version 2.0.13) interrupted the participant to complete computerised VAS ratings (hunger, fullness and pleasantness of the pasta). After consuming 150g, participants were interrupted and provided a fresh dish of 220g of pasta. Participants were asked to eat in this manner until they felt 'comfortably full'. The lunch consisted of pasta shells in a tomato and herb sauce (Sainsbury's UK), served at 55-60ºC (207kcal per 220g serving).

Cookie Snack: Bowls containing 80g of cookie pieces were set on the placemat. Each time the participant ate 10g of cookie pieces, the SIPM software interrupted the participant to complete VAS ratings as described above for pasta. After consuming 60g, participants were interrupted and provided with a fresh bowl containing 80g of cookie pieces. Participants were asked to eat until they felt 'comfortably full'. The cookies were Maryland Chocolate Chip Cookies, with each cookie being broken into 6-7 pieces (390kcal per 80g serving).

Biological samples

Salivary cortisol: Saliva samples were collected and analysed as described for Study 1.

Scanner Task and Stimuli

The scanner task was originally created for use with diabetic patients and consisted of pictures of foods that varied in fat and sugar content (relevant to a diabetic population). The task was run with E-Prime (build 2.0.8.90) in three separate blocks, with each block taking approximately 8 minutes 30 seconds. Each block consisted of 40 food pictures (10 Low Fat / Low Sugar – LL, 10 High Fat / Low Sugar – HL, 10 Low Fat / High Sugar – LH, 10 High Fat / High Sugar – HH), 40 non-food control pictures and 5 smiley face images (see Figure 6.1). Presentation order of food picture types (LH, HF, HS and LL) was fixed, however, the pictures within these sub-groups were presented in random order. For instance, an LH presentation would randomly select one of the 10 available LH images available, without repeating the picture. Each of the three blocks used variations of the same food stimuli (e.g. a different picture of an apple was shown in each block). Stimuli (food + non-food images) were displayed for 2500ms, fixation points were displayed for 3500ms, and smiley face images were displayed for 1500ms. Participants were asked to imagine eating the foods they saw during the task and to press a button on a button box whenever they saw a smiley face to ensure they were maintaining attention. The HH images used high calorie foods (approximately 400kcal per 100g of food shown) and the LL images used low calorie foods (approximately 100kcal per 100g of food shown).

Figure 6.1 Scanner task stimuli consisting of fixation cross, example food images from each food category and smiley face target.

Food Image Ratings

The food ratings task also ran on E-Prime (build 2.0.8.90), and displayed the 120 food images that were shown in the scanner task (30 HH, 30LL, 30 LH and 30 LL). For each image, participants were asked "How appetising does this food look to you?" and "how much do you want to eat this food now?" Participants were instructed to respond as quickly as possible, selecting a number between 0-7 on the keyboard (0 = not at all; 7 = very much).

Procedure

The experimental procedure is summarised in Figure 6.2.

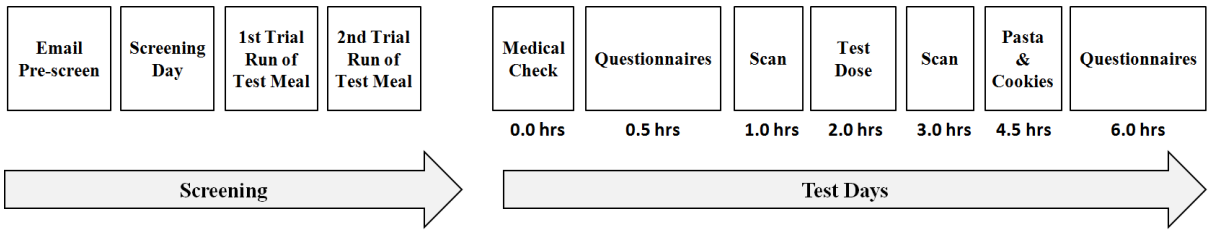

Figure 6.2 Flow diagram for screening process followed by an overview of key events and timings for test days in hours (hrs).

Screening and Test Days:

Screening Days: Participants were sent an email containing: an information sheet detailing the study; key exclusion criteria; and a copy of the TFEQ to complete. Those who met the study criteria were invited to a screening day at the School of Psychology at which they completed a consent form and various screening measures: FSF; Medical Screening Sheet; SCID; NART; EPQ; and a Breakfast and Lunch Questionnaire. Their height and weight was also taken to calculate BMI. Participants returned after a week for two trial runs, both a week apart, with the UEM setup at the WTCRF. Those in the aware group were explicitly told about and shown the UEM balance during each trial, while those in the non-aware condition were not. On both occasions participants ate an ad-libitum lunch of pasta followed 20

minutes later by an ad-libitum snack of cookies (as described above). All participants completed VAS ratings before and after access to each food. These contained mood and appetite items: 'alertness'; 'disgust'; 'drowsiness'; 'light-headed'; 'anxiety', 'happiness'; 'nausea'; 'sadness'; 'withdrawn'; 'faint'; 'hungry'; 'full'; 'desire to eat'; and 'thirst'.

Test Days: Participants arrived at the WTCRF at 9.00am with prior instructions to consume their normal breakfast before arriving. On their first test day, but not their second, they underwent a blood pressure test, electrocardiogram and a physical check with a physician. If a participant passed these checks, they were then breathalysed and completed a pregnancy test each test day, before completing the first batch of questionnaires: Breakfast Form; BDI; BFS; PANAS; STAI; PFS; BIS; and FSF.

Participants also completed baseline VAS. Participants were then escorted to the Birmingham University Imaging Centre (BUIC) for their first scan at 10.00am, after which they completed a set of VAS, provided a saliva sample (all samples were taken using a salivette), and took the test dose (mCPP or placebo) at 11.00am. At 30 minutes post-dosing they completed another set of VAS. After an additional 30 minutes, participants provided a saliva sample and completed VAS immediately prior to their second scan, which occurred at approximately 12.00pm. After completing the scan, participants completed a set of VAS and provided another saliva sample, before being escorted back to the WTCRF.

Immediately prior to lunch at 1.30pm, participants completed VAS and were then given ad-libitum access to a pasta lunch via the UEM; participants in the aware condition were reminded of the presence of the UEM before eating. After finishing lunch, participants completed VAS. This was followed by a 20 minute break after which a further set of VAS

was completed directly before participants were given ad-libitum access to the cookie snack. Immediately after finishing, participants filled out VAS. At 3.00pm (approximately 30-40minutes later) participants completed a second batch of questionnaires: VAS; BDI; BFS; PANAS; STAI; PFS; and BIS. Participants then provided a final saliva sample, rated the scanner task food images, and had a single blood sample taken. Participants returned for a second visit, and the procedure described above was repeated with the only difference being dosing with placebo or drug. At the end of their second session, participants in the non-aware group were questioned to determine whether they became aware of the UEM during the study, were then fully debriefed and given the opportunity to ask questions. They were thanked for their time, and reimbursed for participation.

fMRI Scan

An event-related design was used, utilising the task stimuli described above in pseudo random sequence. A 3.0 T Achieva (Philips) whole body scanner was used at BUIC. T2*weighted echo planar imaging (EPI) slices were acquired every 2.5 seconds (TR = 2.5). 33 axial slices (in-plane resolution of 2.5 x 2.5 x 3 mm - no gap) were acquired, with a matrix size of 96 x 96 and field of view of 240 x 240 mm. 200 volumes were acquired for each block of the task with two dummy scans. A whole brain T2*-weighted EPI volume (resting state) was also acquired, along with an anatomic T1 volume: sagittal plane slice thickness of 1 mm and in-plane resolution of 1.0 x 1.0 x 1.0 mm.

fMRI Analysis

Analysis 1: Effect of placebo versus mCPP on BOLD signals to Low and High Calorie Foods

FSL was used to pre-process and analyse the data as described for Study 5. MELODIC filtered data were entered into a first level analysis, to produce contrast of parameter estimate

(COPE) images for high calorie (high sugar + high fat = HH) and low calorie (low sugar + low fat = LL) food images. These COPES were then averaged across each of the scanning blocks for each participant (i.e. averaging across blocks 1-3 for each scan) in a second level analysis. A subsequent analysis was run on these outputs for each participant to subtract baseline scans from post-dosing scans (i.e. post-placebo minus pre-placebo and post-mCPP minus pre-mCPP). Following this procedure, the outputs were entered into the final mixed effects (FLAME 1+2) group analysis, producing contrasts between placebo and mCPP conditions for BOLD signal activity in response to the low calorie food images and the high calorie food images separately. Group Z statistic images were generated using two level voxel and cluster thresholding to control for error rates (z threshold > 2.3; P threshold < 0.05). To explore the main effect of condition, local maxima are reported. The same FWE correction from Chapter 5 was applied here, with a voxel-wise threshold of $p < 0.001$ (Z > 3.1) only clusters with more than 24 contiguous voxels were significant with a FWE corrected $p < 0.05$. The same adjusted correction was also used for the hypothalamus: $p < 0.0005$; Z>3.3) producing a smaller FWE cluster threshold (19 or more contiguous voxels; p < 0.05). Brain regions were anatomically identified using FSL's integrated Harvard-Oxford Cortical and Subcortical Atlases. For regions not covered by these the Duvernoy Atlas (Duvernoy, 1999) was used. To display brain regions of interest, group Z statistic images were masked using a mask of the brain region (derived from cluster parameters) and then entered into MRIcro (Rorden & Brett, 2000) to visualise BOLD signal % change. This procedure was also used for the analyses below.

Analysis 2: Correlation between BOLD response to high calorie foods and amount of cookies consumed

To examine whether differences in BOLD response to high calorie foods between the placebo and mCPP condition were associated with differences in amount of cookies consumed, correlational analyses were performed. Data were processed as above, up to the final mixed effects group analysis. At this point, each participant's data were entered into individual analyses to produce contrasts for placebo versus mCPP activity for the high calorie food images. Consumption data (total amount of cookies eaten) were also used to produce corresponding difference scores between placebo and mCPP conditions for cookie consumption. The fMRI contrasts were then entered into a mixed effects (FLAME 1+2) higher level analysis with the amount of food consumed differences. Contrasts were produced to investigate positive and negative linear associations between the BOLD signal and these values. Group Z statistic images were generated using two level voxel and cluster thresholding to control for error rates (z threshold > 2.3; *p* threshold < 0.05). Local maxima that survived the FWE correction described in analysis 1 and were correlated with intake data were followed up with 3mm radius spherical ROIs. BOLD percentage signal change values were extracted and correlated with behavioural data using SPSS for visualisation.

Analysis 3: Difference in Baseline BOLD response to High Calorie foods between Responders versus Non-responders

Baseline scan data were pre-processed as described above in analysis 1. The same first and second level analyses as for analysis 1 were then applied to produce high calorie COPES and to average across scanning blocks 1-3 for each scan. These data were then entered into an analysis to subtract placebo day scans from test day scans (i.e. pre-mCPP minus pre-placebo). Following this analysis, the outputs were entered into a mixed effects model (FLAME 1+2),

to produce contrasts between non-responders and responders for BOLD signal activity in response to the high calorie food images. Group Z statistic images were generated using two level voxel and cluster thresholding to control for error rates (z threshold > 2.3; P threshold < 0.05). To explore the main effect of group (responders versus non-responders), local maxima are reported. The FWE correction described for analysis 1 was applied.

Data Analysis

General: One participant was unable to complete both test days due to nausea during the first test day and so their data was removed from all analyses. Two participants were unable to complete both post-dosing scans also due to nausea and to light-headedness; therefore, complete imaging data is only available for 21 participants. For statistical analyses, effects and interactions with condition were examined with analysis of variance (ANOVA). Effects of condition, or interactions with condition, were followed with planned comparisons; Bonferroni correction was used on all t-tests unless otherwise stated. Violations of sphericity were addressed using the Greenhouse-Geisser correction.

UEM: Microstructural data were lost for 7 pasta sessions and 2 cookie sessions due to technical issues, such as participants leaning on the balance, thus analyses were conducted on pasta and cookie data for 17 and 21 data sets respectively. Microstructural data were analysed by quartile, however, as they concord with the overall analysis of UEM data, they are not presented here for conciseness.

6.3 Results

Salivary Cortisol

Cortisol levels at baseline (pre-drug) and immediately prior to the test meal (120mins post dosing) were analysed with ANOVA, which showed no main effects of condition or time

(both $p > 0.05$), but a significant interaction between condition and time (F (1 22) = 8.85; $p < 0.01$). T-tests revealed that there were no baseline differences between mCPP and placebo conditions (4.98 versus 5.59nmol/L; t (22) = -1.14, $p > 0.05$), however, immediately prior to food, cortisol was significantly higher in the mCPP than the placebo condition (6.62 versus 2.77nmol/L; t (22) = -2.62, $p < 0.05$), confirming a response to mCPP.

Subjective state questionnaires:

Questionnaire scores were analysed by within-subjects ANOVAs using condition (placebo versus mCPP) and time (pre versus post dosing) as factors. For the STAI State there was a main effect of condition (F (1 22) = 5.89; $p < 0.05$), with elevated state anxiety in the mCPP (27.72) versus placebo (25.52) condition (see Table 6.1). There was no effect of time (F (1 22) = 1.23; $p > 0.05$) and no significant interaction between condition and time for STAI State (F (1 22) = 3.49; $p > 0.05$). For the BFS there was no effect of condition (F (1 22) = 0.58; $p > 0.05$), but a main effect of time (F (1 22) = 12.10; $p < 0.01$) and an interaction between condition and time (F (1 22) = 5.61; $p < 0.05$). For the interaction, t-tests revealed no significant change from pre-dosing for placebo (11.65 versus 13.61; t (22) = -1.71, $p > 0.05$), but a significant increase in BFS scores from pre-dosing for mCPP (10.52 versus 18.43; t (22) = -3.26, $p < 0.01$), indicating lower mood and energy after dosing. For the following questionnaires and subscales, there were no effects of time or condition, or interactions between time and condition: BDI; BIS; PFS; PANAS POS; PANAS NEG; and STAI TRAIT (all $p > 0.05$).

Table 6.1 Questionnaire Scores for mCPP and Placebo conditions, split by time (standard error of the mean)

	Placebo		mCPP	
Measure	Pre-drug	Post-drug	Pre-drug	Post-drug
BDI	1.38 (0.58)	1.30 (0.54)	2.38 (1.03)	1.74 (0.50)
BIS-11	58.48 (1.71)	58.35 (1.87)	58.75 (1.95)	58.04 (1.92)
BFS	11.65 (3.10)	13.61 (3.02)	11.17 (2.55)	18.43 (3.65)**
PFS	33.09 (2.31)	32.48 (2.36)	33.79 (2.42)	32.52 (2.36)
PANAS-Positive	30.96 (1.85)	31.00 (1.68)	30.79 (1.59)	28.57 (1.75)
PANAS-Negative	10.87 (0.25)	11.09 (0.37)	11.63 (0.76)	11.57 (0.44)
STAI-State	25.78 (1.11)	25.26 (1.09)	26.96 (1.05)	28.65 (1.29)
STAI-Trait	29.65 (1.73)	28.35 (1.46)	31.42 (1.62)	29.87 (1.30)

BDI - Beck Depression Inventory; BIS 11 - Barratt Impulsiveness Scale; BFS- Befindlichskeit scale of mood and energy; PFS - Power of Food Scale; PANAS - Positive and Negative Affective Schedule; STAI - State Trait Anxiety Inventory.
** $p < 0.01$ (difference versus pre-drug)

VAS

Pre-dosing VAS scores were averaged across each item, for a pre-dose baseline. These were then analysed using paired t-tests by condition, which showed no significant differences at baseline (all $p > 0.05$). Post-dosing VAS items were then analysed by condition and time (post-dosing in minutes: *t*30, *t*60, *t*120, *t*150, *t*170, *t*190, *t*200, *t*240). For the following VAS items there were main effects of condition, with elevated scores in the mCPP condition compared to placebo: faint = 8.83mm versus 3.20mm (F (1 18) = 5.08; $p < 0.05$); light-headed = 11.22mm versus 3.32mm (F (1 18) = 13.79; $p < 0.01$); nausea = 8.17mm versus 2.87mm (F (1 18) = 15.68; $p < 0.01$). There were no main effects of time or interactions between condition and time for these items (all $p > 0.05$). For hunger, fullness, desire to eat, alertness, thirst, drowsiness and happiness, there was a main effect of time (all $p < 0.05$), but no effect of condition, or interactions (all $p > 0.05$). For anxiety, disgust, sadness and

withdrawn, there were no main effects of condition or time, or significant interactions (all $p > 0.05$).

To account for the different time durations between VAS questionnaires (which varied more than in Study 1), area under the curve scores (trapezoid method) were calculated for all VAS items. Uncorrected paired t-tests were used. A Bonferroni correction operates on the assumption that tests are statistically independent (these VAS items are highly intercorrelated) and would also have been prohibitively conservative in this instance. T-tests showed increased scores for faint, light-headed, and nausea (all $p < 0.01$), along with decreased hunger and desire to eat (both $p < 0.05$) in the mCPP condition compared to placebo (Table 6.2). All other items were not significantly different (all $p > 0.05$).

Table 6.2 Area under the curve scores for VAS items, split by mCPP and Placebo conditions (standard error of the mean)

VAS Item	Placebo	mCPP
Alertness	14520.22 (892.65)	13252.39 (912.46)
Disgust	601.52 (74.80)	912.17 (198.66)
Drowsiness	3346.52 (672.08)	3450.87 (606.22)
Nausea	606.09 (90.63)	2158.04 (317.22)**
Faint	706.30 (105.49)	2254.13 (582.79)**
Lightheaded	751.09 (121.85)	2938.26 (514.16)**
Anxiety	603.04 (82.50)	717.61 (141.54)
Happiness	15632.17 (447.76)	14662.39 (712.02)
Sadness	740.22 (128.02)	731.74 (128.88)
Withdrawn	787.83 (124.36)	787.17 (170.62)
Hunger	8172.39 (502.31)	6596.30 (718.66)*
Desire to Eat	7830.65 (599.53)	6168.04 (726.58)*
Fullness	10401.74 (650.88)	10176.96 (829.69)
Thirst	4685.46 (582.40)	4594.13 (635.99)

*$p < 0.05$; **$p < 0.01$

Universal Eating Monitor

For all measures of eating behaviour (e.g. amount eaten), each measure was computed for the entire eating episode (e.g. total amount of pasta eaten) and analysed by condition (placebo vs. mCPP 30mg) and food type (pasta vs. cookies) to explore potential interactions. T-tests were then performed for each measure (separately for each food) to explore a priori hypotheses of differential effects of condition on these foods.

Interactions

There was no significant two-way interaction between condition and food type for total amount eaten, time spent eating or eating rate (all $p > 0.05$). However, there was a significant interaction for pauses between mouthfuls (F (1 13) = 10.49; $p < 0.01$). Individual analyses of each measure separated by food type are listed below.

Pasta

Rate of pasta consumption during the meal was significantly reduced in the mCPP condition compared to placebo (t (16) = 2.27, $p < 0.05$, see Table 6.3), and pauses between mouthfuls was significantly increased by mCPP (t (16) = -2.32, $p < 0.05$, see Table 6.3). There was no main effect of condition for total amount eaten (t (16) = 1.43, $p > 0.05$) or for time spent eating (t (16) = -1.43, $p > 0.05$).

Cookies

Participants ate less cookies in the mCPP condition than in the placebo condition (t (20) = 3.09, $p < 0.01$; see Table 6.3), ate at a slower rate in the mCPP condition (t (18) = 4.12, $p < 0.01$, see Table 6.3), and took longer pauses between mouthfuls in the mCPP condition than in the placebo condition (t (18) = -3.82, $p < 0.01$, see Table 6.3). No significant differences were observed between conditions for total time spent eating (t (20) = -0.98, $p > 0.05$).

Table 6.3 UEM measures for Pasta and Cookies, split by placebo and mCPP conditions (standard error of the mean)

	Pasta		Cookies	
UEM Measure	Placebo	mCPP 30mg	Placebo	mCPP 30mg
Amount eaten (grams)	441.0 (45.0)	389.8 (51.1)	38.1 (2.9)	26.6 (3.8) **
Time spent eating (seconds)	402.2 (33.3)	528.2 (70.4)	235.1 (32.6)	269.9 (35.9)
Eating rate (g/min)	64.2 (5.7)	47.7 (6.6) *	11.0 (1.4)	6.6 (1.2) ***
Pause between mouthfuls (seconds)	10.4 (0.8)	15.5 (2.5) *	12.2 (1.7)	25.5 (3.8) **

*$p < 0.05$; **$p < 0.01$;*** $p < 0.001$

Awareness

To investigate whether the awareness manipulation had any effect on eating measures the same analyses above were repeated with the addition of the factor 'awareness' (aware versus non-aware). There were no significant effects of awareness, nor any interactions with condition for any measures (all $p > 0.05$ – see Table 6.4 for means).

Table 6.4 UEM measures for Pasta and Cookies, split by condition and awareness (standard error of the mean)

	Placebo		mCPP 30mg	
UEM Measure	Non-aware	Aware	Non-aware	Aware
Pasta				
Amount eaten (grams)	490.2 (70.7)	400.7 (57.9)	434.8 (65.4)	349.3 (78.2)
Time spent eating (seconds)	382.9 (43.8)	418.0 (50.2)	467.4 (74.5)	582.9 (117.1)
Eating rate (g/min)	73.8 (8.9)	56.4 (6.9)	56.8 (10.0)	39.4 (8.5)
Pause between mouthfuls (seconds)	9.4 (1.1)	11.1 (1.2)	12.1 (1.6)	18.5 (4.4)
Cookies				
Amount eaten (grams)	38.5 (4.4)	37.7 (4.1)	29.7 (5.8)	23.2 (5.0)
Time spent eating (seconds)	221.1 (51.2)	247.8 (43.3)	299.6 (50.4)	237.5 (51.7)
Eating rate (g/min)	13.0 (2.7)	9.1 (1.1)	6.9 (2.0)	6.2 (1.3)
Pause between mouthfuls (seconds)	12.6 (3.4)	11.8 (1.5)	26.7 (4.6)	23.9 (6.8)

Within-Meal VAS Pleasantness Ratings

Within-meal pleasantness ratings for the pasta and cookies were used to calculate mean scores for each eating episode (lunch and snack). Paired t-tests were used to analyse the data by condition. For pasta, there was no significant effect of condition (t (16) = 1.99, $p > 0.05$). However, for cookies there was an effect of condition (t (19) = 2.46, $p < 0.05$), whereby those in the mCPP condition rated the cookies as less pleasant than those in the placebo condition (78.36 versus 84.84mm, see Figure 6.3).

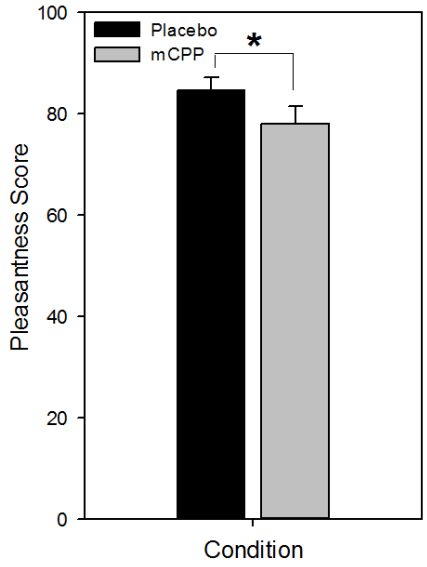

Figure 6.3 Pleasantness ratings for cookie session, split by condition. Pleasantness ratings significantly decrease in whilst consuming cookies, for those in the mCPP condition, compared to placebo. * $p < 0.05$

Correlations between subjective ratings and intake

To investigate the relationship between food intake and subjective ratings (nausea, hunger, and rated pleasantness of the food), correlations were performed on the measures, separately for pasta and cookies, during the mCPP condition. Hunger and nausea ratings were taken directly before the food that was eaten, and pleasantness ratings were used after participants had consumed a first mouthful of the test food. For pasta intake, hunger, but not nausea or

pleasantness, was positively correlated with food intake (r = -0.52, n = 19, $p < 0.05$). For cookie intake, neither nausea nor hunger significantly correlated with amount eaten (both $p > 0.05$), however, there was a trend for a weak positive correlation with pleasantness (r = -0.17, n = 17, $p = 0.07$). In addition, pasta intake did not significantly correlate with cookie intake (r = 0.03, n = 19, $p > 0.05$).

fMRI

Effect of placebo versus mCPP on BOLD signals to Low and High Calorie Foods

The higher level analysis revealed a main effect of condition for activity to both high and low calorie foods pictures, in several key appetitive and reward regions (local maxima are displayed in Table 6.5; local maxima for other regions are displayed in Table 6.6 for reference). mCPP attenuated activity to the sight of high calorie food images in the insula (bilaterally), hippocampus, anterior cingulate, and dorsolateral prefrontal cortex (dlPFC) (Figure 6.4). For the low calorie food images, mCPP bilaterally attenuated activity in the orbitofrontal cortex (OFC) only (Figure 6.5). Dosing with mCPP increased activity to high calorie food images bilaterally in the ventromedial prefrontal cortex (vmPFC) and in the ventrolateral prefrontal cortex (vlPFC) (Figure 6.7), while for low calorie images, mCPP increased activity in the amygdala and midbrain (Figure 6.8).

Table 6.5 Local maxima of key appetitive and reward areas showing main effect of condition (placebo versus mCPP), split by activity to high and low calorie food images

Brain Region (Hemisphere)	Montreal Neurological Institute (MNI) Coordinates			Estimated Brodmann Area	Z-Score
	X	Y	Z		
Reduced activation after mCPP (compared to placebo)					
High Calorie Food Images					
Insula (L)	-42	8	-6	48	3.5
Hippocampus (R)	30	-10	-22	20	3.4
Anterior Cingulate Cortex (R)	2	24	28	24	3.3
Dorsolateral Prefrontal Cortex (R)	32	38	26	46	3.3
Insula (R)	42	10	-10	48	3.1
Low Calorie Food Images					
Orbitofrontal Cortex (R)	22	24	-20	11	3.3
Orbitofrontal Cortex (L)	-16	26	-18	11	3.2
Increased activation after mCPP (compared to placebo)					
High Calorie					
Ventromedial Prefrontal Cortex (R)	20	50	-24	11	3.3
Ventrolateral Prefrontal Cortex (L)	-44	46	-18	47	3.1
Low Calorie					
Midbrain (R)	8	-12	-16	35	3.4
Amygdala (L)	-22	-6	-16	34	3.3

FWE cluster corrected (voxel $p < 0.001$; cluster > 24 contiguous voxels – $p < 0.05$). Left side (L); Right side (R).

Table 6.6 Local maxima of other brain areas showing main effect of condition (placebo versus mCPP), split by activity to high and low calorie food images

Brain Region (Hemisphere)	Montreal Neurological Institute (MNI) Coordinates			Estimated Brodmann Area	Z-Score
	X	Y	Z		
Reduced activation after mCPP (compared to placebo)					
High Calorie Food Images					
Thalamus (R)	16	-26	14	--	4.0
Lateral Occipital Cortex (L)	-40	-86	-10	19	4.0
Occipital Fusiform G (L)	-30	-70	-16	19	3.9
Occipital Fusiform G (R)	26	-76	-16	18	3.9
Temporal pole (L)	-50	10	-8	48	3.8
Temporal G (R)	56	-20	-8	21	3.8
Lateral Occipital Cortex (R)	42	-88	-12	19	3.8
Precentral G (L)	-28	-8	52	6	3.7
Occipital Pole (L)	-28	-90	22	19	3.7
Supplementary Motor Cortex (L)	-6	-4	62	6	3.6
Temporal Occipital Fusiform G (R)	34	-56	-18	37	3.6
Planum polare (L)	-48	-4	-2	48	3.4
Central Opercular Cortex (R)	46	2	10	48	3.3
Thalamus (L)	-16	-24	16	--	3.3
Superior Parietal Lobule (L)	-36	-54	62	7	3.2
Planum Polare (R)	46	-6	-12	48	3.2
Low Calorie Food Images					
Subcallosal Cortex	0	22	-16	11	3.4
Thalamus (R)	20	-24	6	--	3.3
Lateral Occipital Cortex (L)	-38	-64	56	7	3.3
Supplementary Motor Cortex (L)	-2	-8	60	6	3.2
Frontal G (L)	-24	0	68	6	3.2
Increased activation after mCPP (compared to placebo)					
High Calorie					
Angular G (R)	48	-54	42	40	3.2
Lateral Occipital Cortex (L)	-54	-74	26	39	3.1
Low Calorie					
Precuneous (L)	-6	-48	56	5	3.8
Precuneous (R)	6	-46	56	5	3.7
Frontal G (R)	42	4	44	6	3.4
Supramarginal G (R)	68	-38	38	40	3.3

FWE cluster corrected (voxel $p < 0.001$; cluster > 24 contiguous voxels – $p < 0.05$). Gyrus (G); Left side (L); Right side (R).

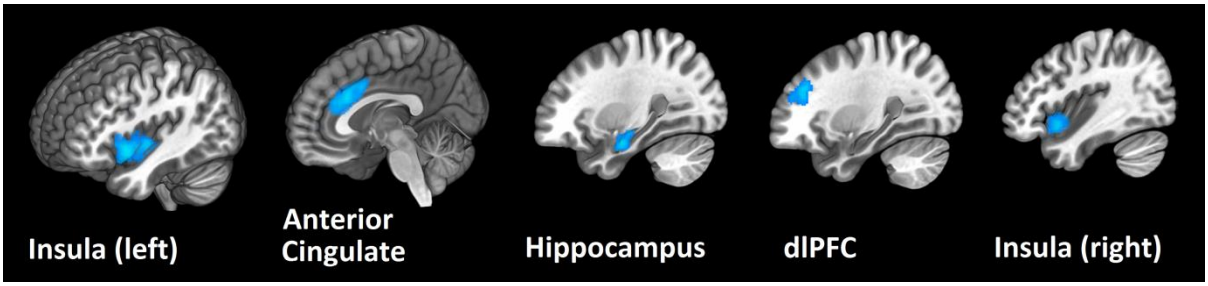

Figure 6.4 Brain areas showing decreased BOLD signal to high calorie food images when dosed with mCPP compared to placebo (dlPFC = dorsolateral prefrontal cortex)

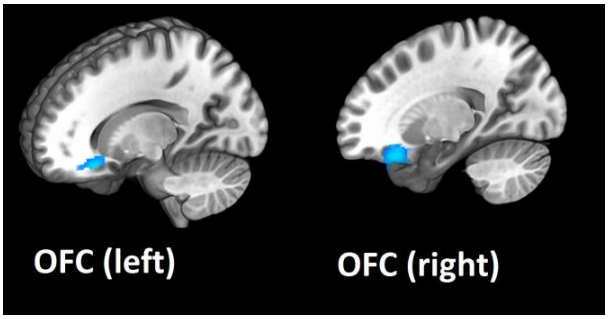

Figure 6.5 Brain areas showing decreased BOLD signal to low calorie food images when dosed with mCPP compared to placebo (OFC = orbitofrontal cortex)

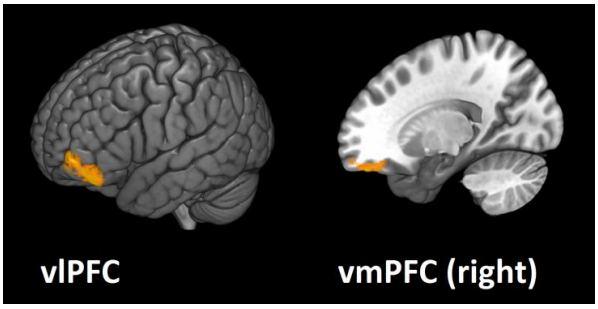

Figure 6.6 Brain areas showing increased BOLD signal to high calorie food images when dosed with mCPP compared to placebo (vlPFC = ventrolateral prefrontal cortex; vmPFC = ventromedial prefrontal cortex)

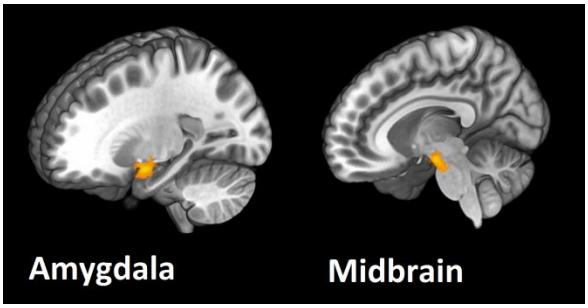

Figure 6.7 Brain areas showing increased BOLD signal to low calorie food images when dosed with mCPP compared to placebo.

Correlations between BOLD response to high calorie foods and amount of cookies consumed

To further examine the relationship between the apparent preferential effect of mCPP on amount of cookies consumed, and BOLD signal activity to high calorie food images, difference scores between the placebo and mCPP condition for amount of cookies consumed and BOLD signals were correlated in a whole brain analysis using FSL. Analyses revealed significant positive correlations between amount of cookies eaten and BOLD signal to the sight of high calorie foods in the orbitofrontal cortex (20, 12, -28; Z = 3.1; r = 0.475, n-20, $p < 0.05$), and ventrolateral prefrontal cortex (34, 64 -8; Z = 3.4; r = 0.552, n = 20, $p < 0.05$) (see Figure 6.8). Although the local maxima for the hypothalamus did not survive the adjusted FWE voxel threshold of $p < 0.0005$ ($p = 0.0013$), a trend for a similar effect was noted (2, 4, -18; Z = 3.0; r = 0.510, n = 20, $p < 0.05$) (see Figure 6.8).

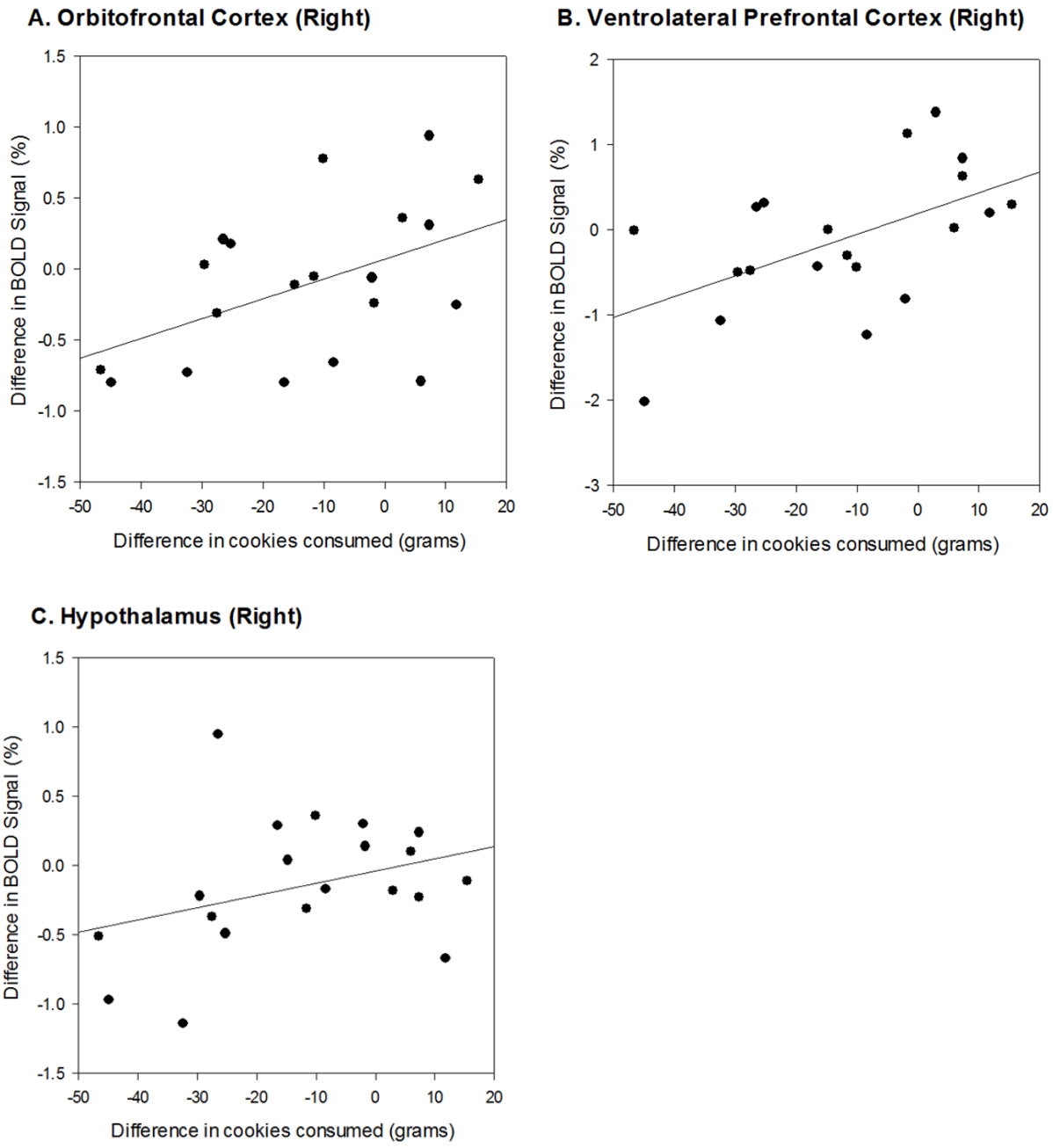

Figure 6.8 Correlations between difference scores for BOLD % Signal and the amount of cookies consumed, between placebo and mCPP conditions. Negative values represent drug induced decreases in BOLD signal and amount of cookies consumed, whereas positive values represent drug induced increases. mCPP attenuated BOLD signal is associated with significant decrease in cookie consumption in the (A) orbitofrontal cortex and (B) ventrolateral prefrontal cortex. A trend was also noted for (C) the hypothalamus.

Responders versus Non-responders Analysis

mCPP had a significant effect on cookie but not pasta intake and therefore analyses of responders versus non-responders was conducted on cookie consumption data. Participants were classified as responders if they showed a > 10% decrease in food consumption after mCPP versus placebo, and non-responders if they showed a < 10% decrease in consumption after mCPP versus placebo. This analysis revealed 12 responders and 8 non-responders.

Behavioural Data

Mixed ANOVA with condition (placebo and mCPP) and response (responder and non-responder) were used to examine cookie intake. There was a main effect of condition (F (1 18) = 14.62; $p < 0.01$), a main effect of response (F (1 18) = 5.77; $p < 0.05$), and a significant interaction between condition and response (F (1 18) = 38.70; $p < 0.001$). Responders ate significantly less cookies after mCPP than placebo (13.9g versus 38.4g; $p < 0.001$), whereas non-responders ate significantly more cookies after mCPP than after placebo (41.9g versus 36.1g; $p < 0.05$).

To explore whether differences in rated hunger or food pleasantness might underlie the differential effect of mCPP on food intake, these measures were analysed with t-tests. Initial ratings for both measures (from the UEM computerised VAS) for the mCPP condition were compared between responders and non-responders. Hunger ratings prior to consuming cookies were not significantly different between responders and non-responders (21.6mm versus 21.1mm; $p > 0.05$). For the rated pleasantness, the non-responder group rated cookies as significantly more pleasant than the responder group (87.6mm versus 70.1mm; $p < 0.05$).

Difference in Baseline BOLD response to High Calorie foods between Responders and Non-responders

During baseline scans (pre-mCPP), cookie non-responders exhibited greater BOLD activity to the sight of the high calorie images than responders across a wide range of key reward/motivational areas, including: orbitofrontal cortex, putamen, posterior cingulate cortex, midbrain, amygdala, parahippocampal gyrus, pons and dorsomedial prefrontal cortex. Brain activity was lower in cookie non-responders (versus responders), only in the ventromedial prefrontal cortex (Table 6.7; Figure 6.9). Other areas showing differences in BOLD signal between responders and non-responders are included in Table 6.8 for reference.

Table 6.7 Local maxima of key appetitive & reward areas showing differences in BOLD signal between non-responders vs. responders to the sight of high calorie food images.

Brain Region (Hemisphere)	Montreal Neurological Institute (MNI) Coordinates			Estimated Brodmann Area	Z-Score
	X	Y	Z		
HIGH CALORIE IMAGES					
Higher activation in Non-Responders vs. Responders					
Pons (R)	6	-28	-26	--	4.0
Midbrain (R)	8	-28	-22	35	3.6
Midbrain (L)	-14	-20	-16	35	3.6
Posterior Cingulate Gyrus (R)	2	-20	36	23	3.2
Amygdala (L)	-24	-10	-12	34	3.2
Putamen (L)	-28	-4	4	--	3.2
Orbitofrontal Cortex (L)	-26	16	-22	38	3.1
Parahippocampal Gyrus (L)	-20	-22	-24	30	3.1
Dorsomedial Prefrontal Cortex (R)	12	64	32	10	3.1
Lower activation in Non-Responders vs. Responders					
Ventromedial Prefrontal Cortex (R)	22	72	-6	11	3.5

FWE cluster corrected (voxel $p < 0.001$; cluster > 24 contiguous voxels – $p < 0.05$).

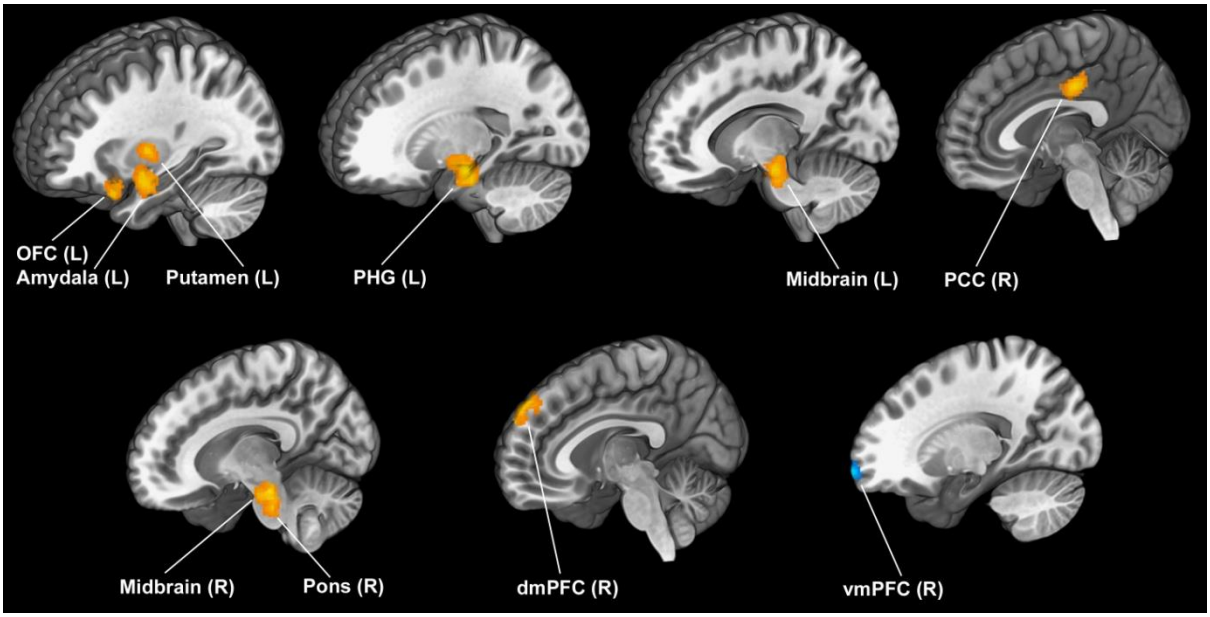

Figure 6.9 Differences in baseline BOLD responses to high calorie food pictures between cookie responders and non-responders. Orange highlights areas in which non-responders showed a greater BOLD signal compared to responders. Blue highlights the single area in which non-responders showed a lower BOLD signal compared to responders. OFC = Orbitofrontal Cortex; PHG = Parahippocampal Gyrus; dmPFC = dorsomedial Prefrontal Cortex; PCC = Posterior Cingulate Cortex; vmPFC = ventromedial Prefrontal Cortex

Table 6.8 Local maxima of other areas showing differences in BOLD signal between non-responders vs. responders to the sight of high calorie food images.

Brain Region (Hemisphere)	Montreal Neurological Institute (MNI) Coordinates			Estimated Brodmann Area	Z-Score
	X	Y	Z		
HIGH CALORIE IMAGES					
Higher activation in Non-Responders vs. Responders					
Temporal Pole (L)	-62	6	-22	21	4.0
Temporal Pole (R)	38	26	-34	38	3.6
Thalamus (R)	8	-2	4	--	3.6
Frontal Gyrus (R)	54	28	-6	45	3.2
Angular Gyrus (R)	60	-50	32	40	3.2
Temporal Occipital Fusiform Cortex (R)	28	-48	-20	37	3.2
Lower activation in Non-Responders vs. Responders					
Superior Parietal Lobule (L)	-44	-46	56	40	3.8
Frontal Gyrus (L)	-30	20	54	8	3.5
Frontal Gyrus (R)	50	30	12	45	3.2

FWE cluster corrected (voxel $p < 0.001$; cluster > 24 contiguous voxels – $p < 0.05$).

6.4 Discussion

The 5-HT_{2C} receptor agonist mCPP at a dose of 30mg decreased salivary cortisol measured 120mins post dosing, confirming activation of 5-HT_{2C} receptors. mCPP reduced ratings of hunger and desire to eat, confirming previously observed effects (see Study 1). However, there was no significant effect of mCPP on pasta intake, which is again consistent with the results of Study 1. In contrast, mCPP induced a significant decrease in cookie intake. The drug also decreased brain activity to the sight of high calorie foods, while exerting a more limited effect on responses to low calorie foods. Correlations between food consumption and BOLD signal showed significant positive associations between high calorie foods and cookie intake whereby the BOLD response to high calorie foods in the orbitofrontal cortex and ventrolateral prefrontal cortex significantly predicted cookie consumption. A further analysis to investigate the behavioural and neural differences between drug responders and non-

responders showed that non-responders showed greater baseline activation across key reward and motivation regions to the sight of high calorie foods than responders. In addition, non-responders showed higher pleasantness ratings of cookies after mCPP than responders.

mCPP attenuated BOLD activity to the sight of high calorie foods in the insula bilaterally. The insula is involved in the sensory processing of appetitive stimuli, and shows reduced activity when individuals report higher levels of fullness (Wang et al. 2008). Therefore, the reduced BOLD signal might represent a neural marker of mCPP induced enhancement of satiation. BOLD activity to the sight of high calorie foods was also reduced by mCPP in the anterior cingulate cortex, an area that has been reported to support goal directed behaviour (Holroyd and Yeung, 2012). Hence, this mCPP-induced effect may reflect reduced motivation to consume the cookies. mCPP unexpectedly decreased activity in the dorsolateral prefrontal cortex, which is implicated in a range of executive functions, including memory and cognitive control (Barbey et al. 2013). Hence, the attenuation may reflect an effect of mCPP on memory processes. However, as the dlPFC is involved in cognitive control, and previous work has related an increase in dlPFC BOLD signal to greater behavioural inhibition (Shackman et al. 2009), the reduction here might represent lowered control, due to a reduced demand for this process. mCPP also reduced the BOLD response in the hippocampus, which is a key area involved in reward and memory. For low calorie food pictures, attenuation of BOLD signals was limited to the OFC (bilaterally) which plays a role in evaluating the subjective pleasantness of food (Kringelbach et al, 2003). Hence, while attenuation of the BOLD response to low calorie images was limited, and primarily related to areas implicated in food liking, reduced BOLD responses to high calorie foods was more widespread, encompassing a number brain regions involved in motivation, sensory processing, inhibitory control and memory.

mCPP also increased BOLD activation to high calorie food images in the vmPFC and vlPFC. The ventral prefrontal cortex is often associated with reward, and activity in the vmPFC has specifically been associated with food reward and goal directed processes (Hare et al. 2009). mCPP increased activation to the sight of low calorie food images in the midbrain and amygdala, both of which are implicated in food reward (Small et al. 2001; Berridge, 1996; Arana et al. 2003). As there is evidence that satiation enhances reward processing in response to low calorie foods (Siep et al. 2009), it is possible that mCPP might cause low calorie foods to be evaluated as more rewarding versus placebo. However, the absence of enhanced pleasantness ratings of the pasta in the mCPP condition suggests this explanation is unlikely.

mCPP at 30mg reduced the rate of pasta consumption by 26% and cookie consumption by 39%, and increased the duration of pauses between mouthfuls of pasta and cookies by 49% and 109%, respectively. Hence, mCPP exerted a greater magnitude of effect on microstructural measures of cookie consumption than pasta consumption. mCPP did not significantly reduce the amount of pasta consumed, which is consistent with the results of Study 1. However, the drug significantly reduced the consumption of cookies.

To assess the possibility that these effects of mCPP were secondary to other effects such as nausea, VAS ratings were analysed. mCPP increased ratings of nausea-like symptoms (nausea, light-headedness and faintness VAS ratings) and reduced mood and energy. However, analysis of nausea ratings indicated that these were not correlated with food intake, suggesting they were unlikely to account for the attenuated eating behaviour, which is consistent with previous findings (Walsh et al. 1994; Study 1). In addition, the differential effects of mCPP on pasta and cookie intake would also suggest the effects are not secondary to nausea as it would be expected that nausea would suppress intake of both foods. Hunger

ratings correlated with pasta intake, and there was a trend for pleasantness ratings to correlate with cookie intake. Thus the pasta consumption might have been more affected by metabolic factors, whereas cookie consumption may have been more influenced by non-homeostatic factors such as hedonic hunger.

The preferential effect of mCPP on cookie eating is interesting and may relate to a specific effect of 5-HT_{2C} agonists on high calorie/palatable foods. In rodents, 5-HT_{2C} receptors mediate the anti-obesity effects of the drug d-fenfluramine (Vickers et al.1999; Clifton et al, 2000). There is evidence that d-fenfluramine selectively reduces fat consumption in rats (Smith et al.1998) and may block a reward system triggered by a high fat diet (Fisler et al. 1993). Similarly, pre-clinical studies with sibutramine, which exerts its effect on appetite at least partly via a 5-HT_{2C} receptor agonist action (Higgs et al., 2011), also demonstrated a reduction in the consumption of high fat food in rats (Popik et al.2011). In humans, dexfenfluramine and sibutramine selectively decreases fat intake (Lafreniere et al. 1993; Rolls et al. 1998). Dexfenfluramine produces a similar effect in healthy men, which is abolished by treatment with ritanserin, a 5-HT_{2C} receptor antagonist (Goodall et al. 1993). Hence, it possible that an agonist action at 5-HT_{2C} receptors is associated with a decreased intake of high calorie foods such as those high in fat, though this is speculative and needs to be tested in future studies. It is also possible that these effects might involve actions at other serotonin receptors.

Participants who were aware of the presence of the UEM did not behave differently to participants who were not aware of its presence indicating that awareness does not affect eating. This finding is consistent with the results of Study 2. Hence, in future studies, it might be preferable and logistically easier to make all participants aware of the UEM setup.

To investigate the relationship between brain activity and food consumption, BOLD responses were correlated with cookie consumption. Cookie consumption was positively correlated with BOLD signals in the OFC and vlPFC. Hence, greater mCPP induced suppression of activity in these areas to high calorie food images, resulted in greater reductions in cookie intake. vlPFC activity has previously been shown to positively correlate with the subjective pleasantness of food consumed (Kringelbach et al, 2003), and thus a reduction in vlPFC activity may be related to a mCPP-induced reduction of the hedonic response to these foods. There was also a trend for a positive correlation between hypothalamic BOLD response and cookie intake. This is particularly interesting as prior work has suggested that the hypothalamic response to sibutramine administration predicts intake of an ad-libitum snack, and subsequent weight change (Fletcher et al. 2010). Thus, these correlations might have utility as longer term indicators of drug induced weight-loss, and should be further explored in future studies.

It is worth noting that in the present study the foods used varied in energy density and palatability. Based on the experimental design it is not possible to conclude whether the effects of mCPP were related to the palatability of the cookies, their relatively high energy density or both. Indeed, there are a number of other differences between pasta and cookies, including their sensory characteristics (salty, sweet, bitter, crunchy, creamy, etc) that may also be related to the preferential effect of mCPP that was observed. In addition, the cookies were served after a satiating meal, and it may be that the preferential effect of mCPP is dependent upon food having been consumed ad-libitum prior to their presentation. For instance, the effect of mCPP on cookie intake might have been observed for any food served after a meal, and this needs to be followed up in future work.

A secondary aim of Study 6 was to examine differences between drug responders and non-responders. Examination of pleasantness ratings showed that after dosing with mCPP, non-responders rated the cookies as more pleasant than responders, in the absence of differences in rated hunger. Analysis of the BOLD signal showed that at baseline (pre-drug), non-responders showed greater BOLD activity than responders to high calorie foods across a range of reward and appetite regions. Taken together, the results suggest that non-responders exhibit greater reward responses to food images and to cookies, and this heightened reward response may explain their difference in response to mCPP. interestingly, Stice and colleagues (2011) reported that healthy individuals at risk of becoming obese, versus those not at risk, show enhanced caudate, putamen, insula, and OFC responses to rewarding stimuli. In addition, it has been reported that the OFC response to rewarding appetitive stimuli predicts subsequent food intake (Nolan-Poupart et al. 2013) and subsequent increases in BMI (Yokum et al. 2011). Hence, it is seems credible to suggest that the heightened reward response might be responsible for a blunting of the hypophagic effect of mCPP on cookie intake.

This study provides the first evidence of a preferential effect of mCPP on BOLD responses to food images and intake of a palatable energy dense snack compared to a pasta meal. These data confirm that the experimental medicine model is capable of detecting distinct neural and preferential behavioural effects, which could help to elucidate the neural and behavioural mechanisms of novel drugs to treat obesity. The marked reduction in activity in reward-related brain areas to high calorie foods could indicate that mCPP and other 5-HT_{2C} receptor agonists may be able to suppress the intake of highly palatable energy dense foods, which would be particularly useful in treating obesity. In addition, investigating drug responders and non-responders has shown that individuals who fail to reduce their cookie intake after

treatment with mCPP show enhanced ratings of cookie pleasantness, and enhanced baseline BOLD signal responses in key reward and appetite areas. The clear differences between responders and non-responders suggests that it may be possible to identify patients who are likely to respond to serotonergic anti-obesity treatments.

CHAPTER 7: General Discussion

7.1. Introduction

The overall objective of this thesis was to develop an experimental medicine model to evaluate the potential efficacy and psychiatric safety of novel anti-obesity drugs. This resulted in three broad aims examining: (1) whether measures of eating behaviour provided by a UEM might have utility as indicators of whether a drug will have a positive impact on eating behaviour and help to elucidate a drug's neural and behavioural mechanism of action; (2) the utility of using measures of emotional processing obtained from the ETB to detect negative psychiatric side-effects; (3) whether using fMRI BOLD signals would provide indications of drug mechanism and whether these measures might prove predictive of eating behaviour. The studies presented in this thesis addressed these questions and the findings from each study will be reviewed briefly to provide an overview of the results. The behavioural and neural markers that emerged and the strengths and limitations of the studies and the model will be discussed. Finally, the broader significance of this work and possible future directions for research will be considered.

7.2. Overview of findings

Chapter 2 investigated whether acute effects of the 5-HT_{2C} receptor agonist mCPP on eating behaviour and emotional processing could be detected using the UEM and ETB in healthy volunteers. It was possible to detect reductions in appetite for a pasta lunch in both men and women which was consistent with previous research (Cowen et al. 1995). However, standard UEM measures such as food intake and eating rate did not show any mCPP-induced alterations. Interestingly, use of the satiation quotient showed that for women, the satiating capacity of the food was enhanced early within the meal by both 15mg and 30mg mCPP.

There were no negative effects of mCPP on any ETB measures, or any other measures of mood and unexpectedly the ETB revealed an enhancement of memory by mCPP. Hence, this Chapter 2 showed that it is possible to detect acute drug effects on appetite and eating behaviour using the UEM and provided a novel finding on memory, illustrating potential for the ETB to detect drug effects on cognitive mechanisms relating to eating behaviour.

A surprising finding reported in Chapter 2 was that a large number of individuals invalidated their test session by interacting with the UEM, e.g. by leaning on the balance or removing their plate from the balance. Chapter 3 aimed to address these issues by examining whether a covert approach was necessary to ensure reliable UEM readings, or whether participants could be made aware of the UEM set up to reduce disruption arising from non-awareness. Chapter 3 also investigated the feasibility of providing a cookie snack 20 minutes after a pasta lunch, to examine whether this could be used in future studies to examine responses to different types of food (highly palatable, energy dense, high calorie cookies versus pasta).

The results from Chapter 3 showed that awareness of the UEM did not affect consumption of a pasta lunch, but significantly reduced cookie consumption. Appetite ratings were affected for both foods, whereby awareness of the UEM decreased ratings of fullness while consuming pasta, and increased ratings of hunger whilst consuming cookies. Overall, the results suggest that the effects of awareness are limited and that use of the UEM without a covert approach should be explored in future studies.

Chapter 2 also provided evidence of a large degree of variability in eating behaviour between participants. One method to reduce such variance is to use a cross-over design, such that

participants act as their own controls. However, the ETB had not been used in cross-over designs and thus, there was a need to examine whether this was a valid approach. Similarly, it was not known whether differences in appetitive state (due to differences in hunger, consuming food, or drug induced changes in appetite) might affect ETB performance, and thereby confound study results. The use of a cross-over design and the potential influence of appetitive state were addressed in Chapter 4. Satiation had no significant effect on any ETB measures. Hence, it seems unlikely that differences in baseline hunger, the consumption of food, or the satiety enhancing effects of a drug would confound ETB test performance. Using a within-subject design with testing over four sessions, it was shown that two of the three memory tasks (ECAT + EMEM) were free from practice effects. However, during the three remaining ETB tasks (FDOT, FERT, EREC), there was evidence of practice effects occurring in the first two sessions, but not the latter two, suggesting that after two initial 'training sessions' to stabilise test performance, it might be possible to use the ETB in cross-over designs.

The aim of Chapter 5 was to profile the brain changes associated with satiation, which could provide a benchmark for comparisons to satiation enhancing drugs. The results showed that satiation decreased BOLD activity across a range of reward areas and increased activity in an area associated with inhibitory control. Further, BOLD activity between prefrontal reward and inhibitory control centres (vmPFC and dlPFC) was correlated, providing novel information on the neural control of appetite and mechanisms governing satiation.

Chapter 6 examined the effects of mCPP on both behavioural and neural responses using a cross-over design. This design was used to address the variance in eating behaviour observed

using an independent groups design noted in Chapter 2. Based on the results from Chapter 3, the effects of mCPP on consumption of both a pasta lunch and cookie snack was assessed in participants who were either aware or unaware of the UEM. Finally, differences between drug responders and non-responders were also investigated. mCPP significantly reduced the eating rate of pasta and cookies and increased the pauses between consumption of both foods. The effect sizes on eating and pauses were greater for cookies, and consumption of cookies was significantly reduced by mCPP whereas there was no effect of the drug on pasta intake. Hence, the model identified a preferential behavioural effect of mCPP on the consumption of a palatable high calorie food. Awareness of the UEM did not affect eating behaviour, suggesting that future studies might benefit from using the UEM in a non-covert design. mCPP reduced brain activity to the sight of the high calorie food images across a range of key appetitive and reward areas, but had a more limited effect on responses to low calorie food images. Hence, the fMRI results mirrored the behavioural results, with more marked effects on the response to high calorie foods images. The fMRI results from this Chapter 6 also overlapped with some of the brain regions indicated in Chapter 5, suggesting potential satiation biomarkers which are reliable across studies. The change in BOLD signal in response to high calorie food images was also shown to be predictive of the amount of high calorie food consumed after mCPP. Thus, investigation of responders and non-responders to mCPP revealed that non-responders showed significantly greater BOLD activity to high calorie food images in reward and appetite regions at baseline than responders. In addition, non-responders showed enhanced rated pleasantness of the cookies. Together these data suggest the novel hypothesis that non-responders to serotonergic weight-loss drugs might have heightened reward responses at baseline which confers insensitivity to the effects of drugs which have 5-HT_{2C} agonist actions (as well as acting at other receptors) such as mCPP.

Furthermore, it is possible that the model might have utility in stratified medicine, as indicator of which individuals are likely to respond to an anti-obesity drug.

In summary, six experimental studies have investigated the potential of an experimental medicine model comprising the UEM, ETB and fMRI to assist with go/no go decisions for anti-obesity drugs undergoing early phase trials in healthy volunteers. The data suggest that the UEM can detect acute and preferential drug effects on eating behaviour. The ETB was able to detect a novel memory enhancing effect of mCPP. Finally, fMRI has been shown to have utility in identifying the neural circuitry underlying satiation and in examining differential responses to distinct types of food stimuli. These measures may also be useful in predicting behavioural responses, and in identifying whether individuals will respond to a given anti-obesity drug. The potential utility of these behavioural and neural markers will be examined in more depth in the following sections.

7.3. Behavioural markers

The behavioural markers of primary interest in this thesis were derived from the UEM and the ETB to measure eating behaviour and emotional processing respectively. The success of these measures across the studies presented in this thesis and their application in the experimental medicine model will be considered here.

7.3.1. UEM Behavioural Markers

The UEM generates several measures of eating behaviour comprising: (1) within-meal appetite ratings (hunger, fullness and desire to eat); (2) measures of meal microstructure (duration of eating, eating rate, number of mouthfuls and pauses between mouthfuls). In addition, using appetite ratings and consumption data it is possible to derive a satiation quotient (SQ).

In Chapter 2 mCPP at doses of 15mg and 30mg was shown to have no effect on within-meal appetite ratings. In contrast, in Chapter 3 it was shown that awareness of the UEM decreased rated fullness while consuming pasta and increased rated hunger while consuming cookies, in the absence of changes in rated pleasantness. This is an important finding, as it shows that within-meal appetite (hunger and fullness) ratings can be dissociated from within-meal pleasantness ratings. In Chapter 6, it was shown that mCPP did not significantly affect within-meal measures of appetite during the consumption of a pasta lunch which is consistent with the results reported in Chapter 2, but significantly reduced the rated pleasantness of a cookie snack. Hence, effects on pleasantness were observed in the absence of effects on appetite, a finding that is similar to previous work with naltrexone (Yeomans and Gray, 1997). Although satiety and reward are tightly interlinked in the human brain (Berthoud, 2011) and it is unlikely that they can be completely dissociated, it is possible that UEM measures could help to indicate whether a drug might have a greater effect on satiation (appetite) or reward (pleasantness). Hence, these measures might be particularly useful in profiling the neural and behavioural mechanisms of drug action.

From this perspective, a simple interpretation of the UEM subjective rating results from Chapters 2 and 6 would suggest that mCPP does not affect appetite and has a selective effect on food pleasantness. This seems unlikely as sibutramine reduces within-meal hunger and increases within-meal fullness (Halford et al. 2010). However, sibutramine also blocks the reuptake of noradrenaline which could account for the disparity. It is worth noting that in both Chapters 2 and 6, mCPP suppression of appetite (via VAS ratings) was observed during the test days, so it seems likely that appetite would also be suppressed during the UEM sessions. Hence, it is possible that mCPP does affect within-meal appetite, but that we were

unable to demonstrate this due to insufficient statistical, power caused by the loss of UEM data.

It is potentially significant that the reduction in pleasantness was preferential for consumption of the cookie snack. mCPP may have a greater effect on higher energy density foods, more palatable foods or foods higher in certain macronutrients and there is previous evidence that serotonin can selectively reduce fat intake (which is linked to energy and palatability) in both rodents and humans (for a review, see Blundell et al. 1995). Indeed, as noted in Chapter 6 there is evidence that the preferential effect is likely due to a 5-HT_{2C} agonist effect on fat intake. However, it is also possible that the effect was due to the consumption of cookies in the absence of hunger; i.e. mCPP might exert a greater effect on food consumed after metabolic needs have been met. Unfortunately, the study design does not enable the dissociation of these factors. It is also possible that the effects of mCPP are mediated by actions at other serotonin receptors. However, it would be useful to explore these issues in future studies.

In Chapters 3 and 6, the experimental manipulations (awareness of the UEM and 30mg mCPP) decreased eating rate whereas no effect of mCPP was observed in Chapter 2. Interestingly, in Chapter 3, the reduced eating rate of cookies was accompanied by changes in within-meal appetite which were reflective of weakened satiety (i.e. increased hunger). Hence, although a decrease in eating rate might be associated with increased satiation (Halford et al. 2010) it has limitations when used in isolation as an indicator of enhanced satiation and ideally should be measured in conjunction with ratings of appetite.

Eating rate is derived from total amount eaten and duration of eating, and the rate can be altered by a change in either one or both of these variables. In Chapter 6, mCPP was observed to have no significant effect on the duration of eating cookies, but a significantly (30%) decreased in the amount of cookies consumed. It is possible that amount eaten and duration of eating reflect the behavioural mechanisms affected by the experimental manipulations. For instance, hunger or non-hedonic influences on intake might be more closely associated with the temporal component of a meal, while food pleasantness might be more closely linked to the amount of food consumed. In support of this interpretation, Yeomans (1996) presented data on the intake and eating rate of a bland versus palatable pasta. Using the data presented in the paper, it is possible to derive the impact of palatability on eating duration and amount consumed. Hence, the bland pasta was associated with a small increase in duration of eating (+2%), but a larger decrease in the amount of pasta consumed (- 11%), which supports the hypothesis that palatability might be more closely linked to amount eaten than meal duration. Further work investigating the effects of satiety and food pleasantness on these meal components in humans would help to clarify this hypothesis.

In addition to eating rate, eating duration and amount eaten, average pause between mouthfuls was also investigated in Chapters 3 and 6. Average pause between mouthfuls was not affected in Chapter 3 by the awareness manipulation, but was significantly increased by mCPP while consuming pasta and cookies in Chapter 6, which might suggest that mCPP reduces motivation to consume both foods.

The within-meal satiation quotient was also examined as a measure of the satiating power of a given amount of food. In Chapter 2, the satiation quotient for pasta was increased during the

first and second quartiles by 30mg mCPP and during the second quartile by 15mg mCPP. These data suggest that mCPP accelerates satiation within a meal. In Chapter 3, participants who were aware of the UEM had lower satiation quotient scores when consuming cookies, than participants who were non-aware of the UEM, suggesting they experienced the food as less filling. Finally in Chapter 6, there were no significant effects of mCPP on the satiation quotient. It is possible that the lack of effect on the satiation quotient in Chapter 6 was due to the difference in paradigm from Chapters. In Chapter 3, participants sat in a test room for 2 hours between dosing and food consumption and periodically completed VAS. In Chapter 6, there was a longer interval of 2.5 hours between dosing and consuming food, as participants were required to walk to and from the scanner room (a 10 minute walk each way) on two occasions and look at food images in the scanner for approximately 1 hour in total (2 x 30 minute sessions). Thus, the Chapter 6 conditions comprised 40 minutes of light exercise and 60 minutes of exposure to food stimuli. Either of these factors may have stimulated appetite, and thereby, attenuated the effect of mCPP on appetite.

It is relevant to consider whether these microstructural measures could be used to detect non-specific drug effects on eating behaviour. For instance, in rats, the behavioural satiety sequence has been used to differentiate behaviourally specific satiety induced by mCPP from non-specific hypophagic effects (caused by locomotor stimulation and stereotypy) induced by the selective 5-HT_{1B} receptor agonist RU-24969 and the 5-HT_{2A} receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (Kitchener and Dourish 1994). Although it was not tested in the current model, it would be beneficial to study the effects of drugs that cause sedation, stimulant effects and/or nausea on eating microstructure to investigate whether characteristic patterns could be identified and subsequently used to detect drugs which attenuate food intake due to non-specific side effects.

Finally, it is important to ask whether the UEM adds anything beyond standard laboratory measures of food intake such as amount eaten. In Chapter 2, mCPP had no effect on amount eaten, but the satiation quotient identified an mCPP-induced acceleration of satiation. In the absence of quartile data for both food intake and VAS, this effect would not have been detectable. In Chapter 6, mCPP reduced the amount of cookies consumed but did not reduce pasta intake. In the absence of additional measures, this may have suggested that mCPP had no effect on pasta intake. However, eating rate was decreased and the duration of pauses between mouthfuls was increased, showing that mCPP affected *how* the pasta was consumed, but not the amount that was consumed. Hence, the ability to examine the structure of a meal, together with the additional measures that are produced by measurement of the temporal profile of an eating episode, provide rich insights beyond that of a single measure of amount eaten. There has been little exploration of the microstructure effects of other drugs that affect eating behaviour. However, it is possible that other drugs also have such within-meal effects, which might be used to distinguish between compounds with different mechanisms of action. For instance, subtle differences between drugs (e.g. on the eating rate or pauses between mouthfuls of cookies) should be detectable by the UEM.

In summary, the UEM is a valuable component of the experimental medicine model, providing rich data regarding drug effects on eating behaviour that might otherwise be missed with cruder outcome measures. The data from the UEM has shed further light on mCPP-induced reductions in food intake, highlighting early within-meal satiation enhancement (Chapter 2), effects on eating rate (Chapters 3 and 6) and preferential effects on highly palatable energy dense foods consumed in the absence of hunger (Chapter 6).

7.3.2. ETB Behavioural Markers

In Chapter 2, the ETB did not detect any anxiogenic effects of mCPP and similarly the questionnaire measures of state and trait anxiety (STAI) also showed no increases in anxiety. While it might have been predicted that mCPP would produce an anxiogenic effect, this is typically observed after intravenous administration, rather than oral dosing (Kahn et al. 1990; Silverstone and Cowen, 1994; Cowen et al. 1995; Anderson et al. 2002b). Hence, it appears that the ETB measures accurately classified oral mCPP as having a safe psychiatric profile at the doses used in Chapter 2. It should be noted however that in Chapter 6, state anxiety was elevated during the mCPP test day and BFS measures of energy and mood were lower after mCPP. The reasons for the discrepancy with Chapter 2 are unclear. It could be speculated that anticipation of being scanned caused mild apprehension for participants, which may have interacted with the drug to produce these effects.

Although no negative psychiatric effects were detected in the current studies, there is previous evidence that the ETB can detect negative psychiatric effects (Horder et al 2009; 2011). Seven days dosing with rimonabant induced a negative bias in memory recognition, which suggests a possible mechanism by which rimonabant induces depressogenic effects (Horder et al. 2011). Furthermore, in a single dose study, rimonabant increased incidental recall of negative information, which also suggests a negative bias (Horder et al. 2009). Hence, the ETB is capable of detecting these adverse effects on mood after single or repeated drug administration.

The ETB might also be useful for assessing other non-specific effects. For instance, some drugs which affect appetite, such as amphetamine, have also been shown to increase alertness

and arousal (Smith and Beecher, 1960). As alertness and arousal are linked to cognitive performance it might be predicted that faster performance on the ETB could be indicative of a drug that increases alertness and arousal and has a general stimulatory effect. Conversely, drugs that have sedative effects and affect eating behaviour (e.g. naltrexone; Oncken et al. 2001) might reduce performance on ETB cognitive measures. This is a particularly important issue, as even in the absence of a negative emotional response (e.g. not showing a depressogenic bias towards negative emotional stimuli), a sedative effect might indicate other symptoms associated with low mood (e.g. tiredness). In a similar approach to that suggested above in relation to the UEM, it would also be interesting to examine how nausea affects responses on the ETB, as it might affect either mood or cognition in a detectable manner.

Investigation of practice effects showed that the ETB can be used in cross-over designs after two practice trials. In addition, the validation study investigating satiety effects showed that neither VAS mood measures nor the ETB task scores were affected by satiation. . Hence, ETB measures are unlikely to be confounded by food consumption which would be particularly advantageous when using drugs which produce large reductions in food intake compared to placebo.

The ETB detected an mCPP-induced enhancement of memory in Chapter 2. This was a novel and unexpected finding and suggests that the ETB might have further utility in eating studies beyond providing an indication of psychiatric safety. For instance, it has been proposed that memory plays an important role in eating (Higgs 2002), and studies in baboons with the memory enhancing drug memantine have shown that the drug enhances satiation (Foltin et

al. 2008). Hence, it is possible that the satiation enhancing effect of mCPP is at least partly due to enhancement of memory.

In conclusion, the ETB provides a sensitive tool to investigate the psychiatric safety of potential anti-obesity drugs. It may also provide the ability to elucidate how a drug affects food intake, particularly by influencing cognitive processes. To this end, it would be particularly advantageous to test the memory enhancing drug memantine using the ETB and UEM and to examine whether any effects on memory are related to any subsequent enhancement of satiation and reductions in food intake.

7.4. Neural markers

In Chapter 5, satiation was observed to decreased neural activity to the sight and taste of appetitive stimuli in the vmPFC, OFC, NAcc (food reward), hypothalamus (energy balance circuitry) and insula (sensory processing), but increased activity in the dlPFC (inhibitory control). These results are important in extending our knowledge of the neural basis of satiation in healthy individuals. For instance, it was unclear from previous studies whether differences in brain activity after a prolonged fasting period (e.g. a 14 hour fast; Führer et al. 2008) would also be present after a shorter period of fasting that would be commonplace between meals for most people. Führer and colleagues (2008) found that satiation (compared to 14 hour fasting) decreased BOLD signals in the amygdala and anterior cingulate cortex to the sight of food images (compared to non-food images), areas which were not significantly attenuated by satiation in the studies of this thesis.

A negative correlation between vmPFC and dlPFC activity was reported in Chapter 5, which raises interesting questions about how reward processes and inhibitory control might interact during satiation. There is evidence that the dlPFC modulates vmPFC activity in response to food stimuli (Hare et al. 2011), which suggests that cognitive (inhibitory) control modulates reward responses. If this is the case, then drugs which enhance inhibitory control might be of use in weight-loss or weight-loss maintenance. Moreover, this would suggest that the dlPFC, and possibly other neural markers of cognitive control such as the dorsal ACC (Mohanty et al, 2007), could be useful indicators of a drug which reduces food intake by this mechanism. Hence, these findings suggest both a novel target for obesity and a means by which to identify drugs with this neural mechanism. It is also interesting to note that the vmPFC-dlPFC negative correlation was observed in satiated but not hungry individuals. It could be speculated that the interaction between these areas was triggered by a bottom-up process; i.e. that it was produced by the metabolic consequences of food consumption. Given that the correlation shows a rise in dlPFC (inhibitory control area) and a decrease in vmPFC (reward area) it is feasible that this interaction then forms the basis of a subsequent top-down influence to limit further food intake. If this were the case these prefrontal areas occupy a pivotal position, both being modulated by, and in turn modulating metabolic consequences. This would be consistent with evidence that there is a significant interaction between top-down and bottom-up influences on eating behaviour (for review see Berthoud, 2011).

Chapter 6 presented a study that investigated the effects of mCPP on BOLD responses to the sight of low and high calorie foods. mCPP at a dose of 30mg decreased activity to the sight of high calorie foods in the insula (bilaterally), anterior cingulate cortex (ACC), hippocampus and dlPFC, but increased activity in the ventromedial and ventrolateral PFC. Conversely, mCPP decreased activity to the sight of low calorie foods in the OFC (bilaterally) and

increased activity in the amygdala and midbrain. Overall, the fMRI results suggest that mCPP had a more wide-ranging effect on brain activity to high calorie, palatable foods, attenuating activity across multiple cortical regions involved in reward and appetite, than to low calorie, less palatable foods. This is consistent with the behavioural data, which showed a more pronounced effect of mCPP on the consumption of high calorie food (cookies) than low calorie food (pasta). The cause of these preferential effects is difficult to determine in the present study, and may be due to differences in a range of factors, e.g.: palatability, energy density, fat content, texture, meal type, etc. Regardless of the precise cause of the preferential effects, the different profiles of BOLD activity for low and high calorie foods confirms the utility of paradigms using different types of food, which may have advantages over the reward-aversion fMRI model used in Chapter 5 for investigating drug effects on specific food categories. Further work is clearly warranted to investigate the precise nature of these preferential effects of mCPP on eating in both lean and obese individuals.

One of the advantages of Chapter 6 was the opportunity to correlate food intake with brain responses to relevant food images (i.e. BOLD signals in response to high calorie food images correlated with amount of cookies consumed). Thus, the magnitude of mCPP induced attenuation of the BOLD signals in OFC and vlPFC to the sight of high calorie foods was correlated with the magnitude of the decrease in cookie intake caused by the drug. There was also a trend towards a correlation when considering the hypothalamus. Thus, while mCPP did not affect the mean hypothalamic response to food images, there is an association between the magnitude of the mCPP effect on hypothalamic response and on cookie intake. This is consistent with the work of Fletcher and colleagues (2010) showing that the magnitude of the effect of sibutramine on hypothalamic responses, correlated with ad-libitum eating and weight over time. Together with the correlations with the prefrontal reward areas, the data

suggest that energy balance circuitry in conjunction with food reward regions mediate the effect of 5-HT_{2C} receptor activation on the amount of food consumed. Hence, the BOLD signal in these regions is predictive of food intake, and may provide a useful longer term indicator of weight change, though this remains to be investigated further.

mCPP attenuated BOLD response in the hippocampus, an area which is implicated in reward. The ventral hippocampus is particularly noted for connectivity with the NAcc and VTA in humans (Kahn and Shohamy, 2013). Hence, it is likely that the hippocampus is part of the wider reward circuitry affected by mCPP. It is also interesting to note that the hippocampus is a key memory centre, and that mCPP was observed to enhance memory in the studies reported in Chapter 2. Unfortunately, measures of memory were not taken in Chapter 6; nevertheless, it is tempting to speculate that the hippocampal BOLD modulation might be related to the cognitive enhancing effect of mCPP.

ACC attenuation in Chapter 6 is likely to represent a decrease in motivation and attention towards the food presented, while dlPFC attenuation might indicate a decrease in inhibitory control. The dlPFC and ACC are functionally connected to one another, and interact to exert cognitive control over behaviour (MacDonald et al. 2000; Cieslik et al. 2013). Hence, it is possible that mCPP attenuation of ACC attention and motivation to food stimuli reduced the need for dlPFC control, resulting in a decrease in dlPFC activity. However, this is speculative and requires investigation.

Both natural satiation and mCPP attenuated activity in the insula and the OFC (see Chapter 5 and 6). This raises the possibility that the effect of satiation in Chapter 5 was driven, at least

in part, by an agonist action of endogenous 5-HT at 5-HT_{2C} receptors, as these brain regions express a high density of 5-HT_{2C} receptors (Abramowski et al. 1995; Sharma et al. 1997). A caveat is that the use of different scanner tasks might be expected to yield different patterns of activation, regardless of any differences due to natural satiation and mCPP- induced satiation. Nevertheless, it is also possible that any disparity in results between these studies may reflect differences between how mCPP and natural satiation affect the brain. If this were the case, then natural satiation may primarily attenuate hypothalamus and NAcc activity, whereas mCPP may primarily attenuate activity in the dlPFC (inhibitory control), ACC (motivation) and hippocampus (memory).

Both the work presented in Chapters 5 and 6, and the results of Fletcher and colleagues (2010) show that the NAcc response to food is attenuated by satiation with food, but not by sibutramine or mCPP, which suggests that 5-HT_{2C} receptor stimulation does not directly affect this key reward structure. Perhaps more surprising is that mCPP did not affect hypothalamic activity (Analysis 1 in Chapter 6), as the hypothalamus is a 5-HT_{2C} rich area in humans (Marazziti et al. 1999). Sibutramine has been shown to decrease hypothalamic activity (Fletcher et al. 2010) while intravenous dosing with mCPP has been shown to immediately increase the hypothalamic response (Anderson et al. 2002b; McKie et al. 2011). It is possible that the additional noradrenergic effects of sibutramine contribute to its effect on the hypothalamus, and that the increased response seen immediately after intravenous administration in the scanner, is not present 60 minutes after oral dosing. Also, as BOLD changes in the hypothalamus can be difficult to detect and the hypothalamus is a heterogeneous region, the lack of effect may also be due to technical limitations of our studies.

Satiation was shown to enhance dlPFC activity in Chapter 5 and mCPP was observed to enhance ventral PFC, amygdala and midbrain activity in Chapter 6. These areas are associated with motivation and liking (Small et al. 2001, Berridge, 1996). Previous work has noted that satiation can enhance reward processing in response to less energy dense foods (Siep et al.2009). However, as pleasantness ratings of the pasta were not enhanced by mCPP, it may be that these increased activations are not representative of reward enhancement.

Collectively the results suggest that mCPP does not affect core reward and appetite areas in the same manner as natural satiation (NAcc and hypothalamus), but primarily exerts its influence more comprehensively across a range of areas implicated in reward (see Figure 7.1).

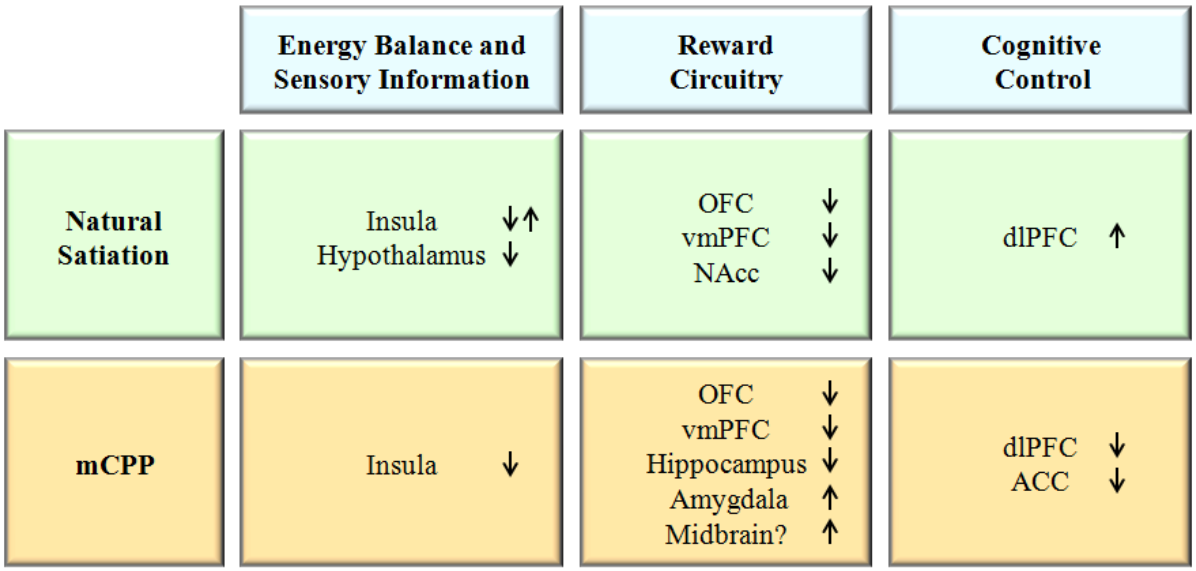

Figure 7.1 Model of natural satiation and mCPP-induced changes in BOLD signals to appetitive stimuli (based on the results described in Chapters 5 and 6).

The neural profile of mCPP points more strongly towards modulation of reward circuitry than energy balance circuitry (compared to natural satiation) and this is supported by effects on food pleasantness (but not hunger or fullness) within meal reported in Chapter 6. If the primary effect of mCPP is on the rewarding aspects of food, it is interesting to consider which processes may be directly affected. One possibility is that mCPP enhances alliesthesia. Alliesthesia refers to the phenomenon whereby eating food is more pleasant when an individual is hungry and less pleasant when they are satiated. Thus, food pleasantness is dependent upon the internal state (Cabanac, 1971). It is possible that mCPP enhances alliesthesia such that food becomes less pleasant at a faster rate during the meal, thereby accelerating satiation. The early effect of mCPP on the satiation quotient reported in Chapter 2 lends support to this theory. In addition, cyproheptadine is an antagonist at 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, and increases food intake and significantly reduces alliesthesia in humans (Silverstone and Schuyler, 1975; Fantino et al. 1990). Hence, it seems plausible that decreased food intake induced by serotonergic agonists might be due to enhanced alliesthesia. However, to date, the only research which examined the effects of enhanced 5-HT function (by administration of dexfenfluramine) on alliesthesia reported no significant effects (Blundell and Hill, 1988). A study that examined the effects of mCPP or another 5-HT_{2C} receptor agonist in an alliesthesia paradigm would provide an important test of this hypothesis.

The final application of fMRI within this thesis was the exploration of drug responders and non-responders. During their pre-drug baseline scan, non-responders (compared to responders), showed increased BOLD responses across multiple brain areas involved in reward and motivation to the sight of high calorie foods. Hence, while mCPP and other 5-HT_{2C} agonists such as lorcaserin might not be particularly effective for treating obesity in

these individuals, perhaps drugs which are known to target other neurotransmitter reward mechanisms (e.g. naltrexone) would be more useful and this would be interesting to test in this model. Thus, the responder versus non-responder approach could be useful for stratified medicine as an investigative tool to identify the appropriate drug for individual subjects during clinical trials, and possibly in the future, as a diagnostic tool in the clinic to identify the drug to which a patient is most likely to respond.

In conclusion, the data from this thesis suggest that experimental medicine models that incorporate fMRI measures may be useful in identifying drugs at an early stage of development that may be useful in treating obesity and in identifying individuals who might best benefit from particular drug treatments. Further work to assess the relationship between BOLD responses to food images and weight change over time would greatly enhance the potential utility of fMRI within an experimental medicine model.

7.5. Strengths, limitations and future work

To assess the strengths and limitations of the experimental medicine model that has been developed in this thesis, it is useful to consider objective criteria. In a recent review, Harmer and colleagues (2011) suggested that experimental medicine models should be able to meet five requirements. First, they must respond to conventional drug treatments. Second, similar effects should be observed in healthy volunteers and clinical populations, meaning the model must be translational. Third, the model should be sensitive to drugs operating via different pharmacological mechanisms and be able to discriminate between them. Fourth, they should not be sensitive to failed or ineffective drugs, to allow go / no go decisions to be made.

Finally, the model should be sensitive to bi-directional results (e.g. changes in either direction, such as in this case increased or decreased food intake).

It was not possible to directly test the first criterion (that the model must respond to conventional drug treatments) within this thesis due to the lack of available licensed centrally acting anti-obesity drugs to test. However, the recent approval of lorcaserin, Qsymia and Contrave by the FDA could change this landscape. As mCPP has been used in many studies over a number years to assess appetite and food intake in rats and humans (Kitchener and Dourish 1994; Kennett et al. 1994; Hewitt et al. 2002; Walsh et al. 1994; Sargent et al. 1997), it could be argued that the studies with mCPP in this thesis has provides evidence to start to address the first criterion.

In addition, previous work has shown that the UEM is capable of detecting the effects of sibutramine on eating behaviour (Barkeling et al. 2003; Halford et al. 2010); the ETB can detect negative psychiatric effects of rimonabant (Horder et al. 2009; Horder et al. 2011) and fMRI can be used to detect the effects of sibutramine (Fletcher et al. 2010) and rimonabant (Horder et al. 2010). Hence, there is reasonable evidence that the proposed model can fulfil the first criterion.

The second criterion is that similar effects should be observed in healthy and clinical populations. However, the model was exclusively validated with healthy volunteers in the studies that comprise this thesis and hence future work is required with clinically obese patients to ensure that results are translational. This is particularly important as there are known differences between lean and obese participants in eating behaviour measured by the

UEM (Laessle et al. 2007), responses to food stimuli measured using fMRI (for review see Carnell et al. 2012); and in cognition and prevalence of depression which might be detectable with the ETB (Smith et al. 2011; Carey et al. 2014). In addition, it is not known whether using the UEM in a non-covert manner might affect the eating behaviour of obese individuals, to a greater extent than their lean counterparts. Hence, by incorporating acute testing of clinical groups alongside healthy controls in a future study, it would be possible to assess whether results are translational.

The third criterion (the model should be sensitive to drugs operating via different pharmacological mechanisms) has not been directly addressed in this thesis. However, we have shown that the UEM, fMRI and ETB are sensitive to other experimental manipulations. Nevertheless, a key question is whether the model can differentiate between different drugs. Unlike mCPP, sibutramine has been shown to reduce the total amount of pasta consumed in UEM studies (Halford et al.2010; Barkeling et al. 2003). In addition, UEM work by Yeomans and Gray (1997) showed that naltrexone produces a differential response to mCPP, reducing total intake and rated pleasantness of pasta. Further, Horder and colleagues (2009; 2011) showed that rimonabant has a contrasting profile to mCPP in the ETB inducing a negative memory bias. Finally, there is fMRI evidence that sibutramine produces different effects to mCPP on BOLD responses (attenuating hypothalamic and amygdala responses). Hence, it has been independently shown that each component of the model responds to different drugs with different mechanisms to produce distinctive effects. However, it would still be instructive in future to compare different drugs within a single study. Although there were no centrally acting licensed drugs available for obesity in the UK while these thesis studies were being conducted, the recent approval of lorcaserin, Qsymia and Contrave may allow for such a study.

The fourth criterion is that the model should not be sensitive to failed or ineffective drugs. Although the withdrawn drug rimonabant was tested in an fMRI paradigm and the ETB and showed a profile that was consistent with the reason for its withdrawal from the market (Horder et al. 2009; 2010; 2011), the UEM has not been tested with 'failed or ineffective drugs' and this could usefully be investigated in future work.

The final criterion is that the model should be bi-directionally sensitive. In the fMRI studies, increased and decreased BOLD activity in response to food stimuli was reported in Chapters 5 and 6. The changes in UEM measures were largely uni-directional and so it would be useful to test a drug which enhances appetite and food intake. While the studies in this thesis did not show bi-directional results for all of the ETB measures, there are a wealth of studies that have reported such changes (Chapter 1 – Table 1.1). Hence, with the exception of the UEM, the model has been proven to be capable of detecting bi-directional results.

Therefore, in relation to Harmer and colleagues original five criteria: (1) each element of the model has been shown to be responsive to an anti-obesity drug, and to mCPP within this thesis; (2) the model remains to be translated into obese populations in future work, and this should be done as a matter of priority; (3) each element of the model has been shown to be sensitive to drugs operating via different pharmacological mechanisms, and the testing within this thesis has contributed to this body of data; (4) further investigation of 'ineffective' agents is required; (5) the ETB and fMRI have demonstrated a capacity for detecting bi-directional results but further testing of appetite enhancing drug would help confirm this ability for the UEM. Thus, overall, the model is performing in line with the majority of these criteria. The most immediate priority for validation would be to replicate these studies in an obese population.

It is also worth noting that the model was validated with healthy women in the majority of studies in this thesis (4 out of 6). The focus on women was because men exhibited unusual eating behaviour (eating very large amounts of food) during the study reported in Chapter 2. It is unclear at present whether the results reported from the UEM studies in Chapter 3 would extend to male participants, although it appears unlikely that such effects would be gender specific. The majority of work examining awareness has focussed exclusively on female participants (Polivy et al. 1986; Roth et al. 2001; Robinson and Field, 2015). However, there is evidence that gender influences other social phenomenon such as conformity, whereby males are less likely to conform than females (Eagly et al. 1981). Hence, further examination is required to clarify whether gender interacts with awareness.

It is possible that the UEM results reported in Chapter 6 might fail to translate to male participants, based on the disparity between male and female eating profiles observed in Chapter 2. Indeed, there is evidence that in rodents that mCPP has a greater hypophagic effect in females than males (Clifton et al. 1993). In humans, mCPP takes longer to reach peak plasma levels in females (Cowen et al. 1995) and dexfenfluramine (which releases 5-HT and inhibits reuptake by the 5-HT transporter) induces greater prolactin responses in females compared to males (McBride et al. 1990). Hence it is possible that females may be more sensitive to serotonergic manipulations, and that any effects in males may be smaller in magnitude. Thus, the UEM component of the model will need to be validated with men, particularly as safety data for compounds in the early stages of clinical development may only enable studies in males. Future studies may need to exclude men who eat excessive amounts of food or exhibit unusual eating patterns to enable the collection of robust data.

In the ETB studies, the absence of gender effects (Chapter 2) suggests that gender is unlikely to influence results. Similarly, in the natural satiation fMRI study (Chapter 5) the lack of gender effects suggests this is also a suitable endpoint for both males and females. However, recent data suggest that fMRI BOLD signals as a measure of brain reward can be influenced affected by the stage of the menstrual cycle (Dreher et al. 2007). Thus, it is recommended that this factor is controlled for in future experimental studies.

It is also worth noting that while the effects of mCPP on appetite and food intake are likely to be mediated by agonist actions at 5-HT_{2C} receptors, it is possible that agonist actions of the compound at other serotonin receptor subtypes could also contribute to these effects. To help clarify this situation in humans, it would be useful to examine the effects of a more selective 5-HT_{2C} receptor agonist such as lorcaserin on appetite and food intake in the UEM model.

A final, more general, point concerns the use of fMRI. While the method has clear utility, it is worth noting there are some limitations to this technique. Firstly, the use of different paradigms and different analysis techniques has the potential to yield different results. Hence, it is important to standardise analysis procedures, and this has been the case within this thesis wherever possible (i.e. consistent use of FSL, pre-processing techniques, threshold values, local maxima exploration and FWE correction). A second point concerns the attribution of function to a neural correlate. As an example, in Chapter 5 we observed an increase in activity in an inhibitory control area (dlPFC). However, this does not imply that inhibitory control was measured in the study. Instead, based on previous published findings we were able to identify the dlPFC as an area involved in inhibitory control, and speculate that modulation of response in this area could represent a change in this function. The third point

concerns interpretation of the directionality of a result. To clarify, in the non-responder analysis we observed differential BOLD signal responses between mCPP responders and non-responders. It was concluded that non-responders showed greater reward-related activity compared to responders. However, it could also be concluded that the responders showed lower reward activity when compared to responders. We speculate, based on previous studies, that the former interpretation is a better fit with current theories of reward processes. In addition, it is important to note that an increase or decrease in BOLD signal does not necessarily imply an increase or decrease in functional activity either, only a change or difference from baseline. Hence, inferring directionality should be treated with caution and the limitations of the conclusions considered carefully.

In terms of the future direction of the model presented in this thesis, several suggestions, and future studies have already been outlined above and a number of others are considered below. For instance, structural MRI data were collected in the studies described in Chapter 6 and hence, it will be possible to explore potential structural differences between drug responders and non-responders.

An exciting future addition to the model would be to examine the role of genotypes in drug responding. For instance, polymorphisms in SLC6A2 and GRIN1 (associated with noradrenaline and NMDA, respectively) are associated with increased weight-loss in a sub-group of patients treated with GW320659 (Spraggs et al. 2005). This suggests that genotyping could have utility in identifying drug responders versus non-responders, or even populations that show a maximal therapeutic response. There is evidence from pharmacogenetic studies that this work can be translated to serotonergic drugs, as

polymorphisms in the serotonin-transporter-linked polymorphic region (5-HTTLPR) are associated with differential responses to sibutramine (Vazquez Roque et al. 2007; Grudell et al. 2008). Similarly, polymorphisms of the HTR2C gene are associated with body weight (Bah et al. 2010). Hence, it seems plausible that a genetic exploration of the response to serotonergic drugs, in addition to other anti-obesity drugs, could significantly increase the ability of the model to detect drug responders versus non-responders.

7.6. Concluding remarks and significance

In conclusion, the work in this thesis has contributed to the development of an experimental medicine model for testing of anti-obesity drugs. The studies described provide evidence that the UEM can be used in non-covert within-subjects designs to detect preferential drug induced effects on eating behaviour and provide information on drug neural and behavioural mechanism. The ETB is also sensitive to single dose drug- induced effects, can be used in within-subjects designs, is robust to changes in appetite and satiety, and may also provide information on behavioural mechanism. Finally, fMRI represents a valuable tool to examine responses to a variety of appetitive stimuli and to understand the neural circuitry and behavioural mechanisms involved in drug response. It may also be particularly valuable for its predictive utility regarding individual responses to drugs.

The results shed light on a number of interesting psychological and pharmacological issues. mCPP was shown to affect memory, which raises the interesting question of whether memory enhancement might facilitate satiation and ultimately weight-loss. mCPP was also shown to have a preferential effect on cookie intake, which might be particularly advantageous in combating the consumption of energy dense foods in treating obesity. Investigation of natural satiation has provided a comprehensive neural profile of the process that mediate eating

behaviour., The results identified the involvement of multiple brain regions, including energy balance and reward circuitry in the control of appetite in healthy human volunteers.

This thesis has also raised broader issues such as the nature of drug response and non-response. This issue has significance that extends beyond anti-obesity drugs and may apply to most if not all drugs used to treat psychiatric disorders. It seems likely that in the years ahead, medicine will become increasingly personalised and as such, research will need to take this into account and adjust to be able to detect the finer grains of drug response. It is hoped that this line of thinking, and the data in this thesis, will assist in the much needed re-calibration of anti-obesity medication towards a stratified approach.

While there are limitations to the work presented in this thesis, implementation of suggestions for future work should enable the validation of a robust experimental medicine model with the capacity to successfully identify safe and promising anti-obesity drugs. The most pressing area is the validation of the model for clinical populations to ensure that results translate to obese patients. The model would also benefit from testing other drugs with different mechanisms, to investigate the model's ability to differentiate between them and to further investigate whether they possess preferential effects which have not been detected to date. The incorporation of genotyping would further augment the ability of the model to investigate populations who will benefit most from a given drug, and might ultimately lead to the most swift and economically efficient method of identifying which drug a patient is likely to respond to.

This thesis opened with a consideration of the urgent need for new treatments for obesity. That need is still pressing, and a robust neuropsychopharmacological approach can play a

pivotal role. This model presents a novel opportunity to enhance the development of anti-obesity drugs and to contribute to stratified medicine, in a way that could greatly enhance the treatment of obesity.

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