Neural Substrates of Feeding Behavior: Insights from fMRI Studies in Humans

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INTRODUCTION

The main objective of the present thesis was to investigate the neural response to visual food and non-food cues in humans using functional Magnetic Resonance Imaging. This response was measured following two different feeding-related protocols: (1) the administration of the appetite-stimulating peptide ghrelin and (2) the manipulation of cognitive expectation of food. Given the increasing rates of overweight and obesity in Canadian Society and across the globe, there is much scientific interest in understanding how the brain controls appetite and nutrient consumption, thereby constituting the rationale for the present research studies.

The format option selected for this work is the manuscript-based dissertation. For purposes of clarity, the thesis is organized into three modules, with a total of six chapters. Module I overviews the scientific literature, Module II introduces the experiments, and Module III offers an integrative discussion. The pre-module prefaces bridge the three sections, and study-specific hypotheses are stated in Module II. Contents of each chapter are summarized below.

MODULE I: LITERATURE REVIEW

CHAPTER 1: FEEDING

This chapter examines homeostatic and extra-homeostatic feeding. Key neural structures associated with these two types of feeding are discussed; as well, evidence demonstrating an interaction between homeostatic factors/ substrates and reward circuitry is presented.

CHAPTER 2: GHRELIN

This chapter covers the hormone ghrelin. A spectrum of topics are reviewed including, the sources, receptors, and clinical pharmacokinetics of the peptide, as well as its involvement in nutrient regulation, reward and memory.

CHAPTER 3: NEUROIMAGING

This chapter introduces neuroimaging with a focus on functional Magnetic Resonance Imaging. In addition to the technical background, published studies having employed imaging as a tool for the assessment of feeding behavior are described.

MODULE II: EXPERIMENTS

CHAPTER 4: RESEARCH STUDY I

This chapter presents the first manuscript entitled *"Ghrelin Modulates Brain Activity in Areas that Control Appetitive Behavior"*. This study mapped the neural response to visual food and non-food images in healthy male subjects, following intravenous ghrelin administration, with functional Magnetic Resonance Imaging.

CHAPTER 5: RESEARCH STUDY II

This chapter presents the second manuscript entitled *"State of Expectancy Modulates the Neural Response to Visual Food Stimuli in Humans"*. This project evaluated the cerebral response to visual food and non-food pictures during the conditions of 'expecting' food and 'not expecting' food, in fasted male participants, using functional Magnetic Resonance Imaging.

MODULE III: GENERAL DISCUSSION

CHAPTER 6: PERSPECTIVES

This chapter discusses the experiments presented in Module II. Similarities between the projects, clinical implications and methodological limitations are examined, as well, main findings of the thesis are considered with respect to the popular model of energy homeostasis. Future directions for appetite-related imaging research are also proposed.

STATEMENT OF ORIGINALITY

In compliance with McGill Faculty of Graduate Studies and Research guidelines, "elements of the thesis that are considered to constitute original scholarship and an advancement of knowledge in the domains in which the research was conducted must be clearly indicated". I hereby attest that the two studies presented in Module II represent novel endeavors that contribute to the progress of science in the field of feeding regulation by the central nervous system.

Feeding is a multifaceted phenomenon that has yet to be fully understood in man. The scope of this thesis was to broaden the comprehension of ingestive processes from two alternate perspectives using functional Magnetic Resonance Imaging paired with visual food and non-food cues. Data acquired from these investigations, along with associated interpretation of results, comprise original scholarship that has been translated into two scientific publications.

CONTRIBUTION OF AUTHORS

As per university regulations, a thesis containing multi-authored papers mandates an explicit statement indicating the contribution of each individual. This information for the two manuscripts presented in the current dissertation is provided below.

PUBLICATION 1:

Ghrelin Modulates Brain Activity in Areas that Control Appetitive Behavior. (2008). Malik S, McGlone F, Bedrossian D, and Dagher A. Cell Metabolism, Volume 7 (Issue 5): Pages 400-409.

This manuscript describes the effects of intravenous ghrelin infusion on the neural response to visual food and non-food stimuli. The first author (S Malik) designed and executed the study, in collaboration with the last author (A Dagher). As ghrelin is not approved for administration to humans in Canada, Therapeutic Products Directorate approval was required. Preparation and submission of a Clinical Trial Application to Health Canada was mainly the responsibility of the first author (S Malik). Under the supervision of A Dagher, data acquisition and analysis were also predominantly carried out by the first author. Blood sampling and ghrelin infusions were performed by A Dagher. Supplementary information included in the paper, were extracted from an independent study completed by D Bedrossian, under the mentorship of A Dagher and S Malik. The original draft of

the manuscript, composed by S Malik, underwent editorial processing by A Dagher. The second author (F McGlone) offered useful insight regarding the paper.

PUBLICATION 2:

State of Expectancy Modulates the Neural Response to Visual Food Stimuli in Humans. (2008). **Malik S**, McGlone F and Dagher A. Submitted to NeuroImage.

This manuscript describes the effects of food expectancy on the cerebral response to visual food and non-food stimuli. The concept of the current experiment was proposed by A Dagher. All aspects of data collection, analysis, and interpretation were primarily conducted by S Malik, with assistance from A Dagher. Multiple drafts of the article (written by S Malik) were critically reviewed by A Dagher, with each subsequent version incorporating suggested comments. F McGlone contributed to the manuscript.

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The pursuit of this degree has been a remarkable experience. My knowledge of neuroscience, brain imaging and clinical research has grown enormously, even beyond my own expectations. This journey however, would not have been possible without the support and guidance of numerous people.

I would like to begin by recognizing and thanking my PhD supervisor, **Dr. Alain Dagher**. He accepted me into his lab and provided much-needed research advice and expertise over the course of my graduate studies. Not only did he teach me a tremendous deal about imaging, feeding, and addiction, but was always more than willing to answer my questions, and provided extensive editorial assistance with both the thesis and the manuscripts. His mentorship has significantly shaped my scientific thinking, and has also afforded me direction for the future, for which I am grateful. I am also very appreciative of his encouragement to attend conferences, both local and abroad. The meetings were fantastic experiences and provided great incentive to persevere. Overall, the continuous assistance and understanding provided by my supervisor has greatly contributed to the successful termination of my PhD research.

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Due to the elaborate nature of our *ghrelin study*, extra-departmental collaboration was required. I would like to thank **Dr. Louise Thibault**, an associate professor in McGill University's School of Human Nutrition and Dietetics, for her assistance in the design of the standard test breakfasts employed in our research study. I am also extremely grateful to **Dr's Hinko Nedev** and **Uri Saragovi** at the Lady Davis Institute (Jewish General Hospital, McGill University Health Center), for their assistance with the HPLC stability testing of our peptide. Finally, many thanks go to **Dr. Keith Worsley** (Department of Mathematics and Statistics, McGill University), for his help with statistical issues, as well as for his multiple attempts to resolve some of our complex functional MRI data.

I would like to thank a number of individuals in the Department of Neurology & Neurosurgery. In particular, my PhD advisory committee members, **Dr. Marilyn Jones-Gotman** and **Dr. Sonia Lupien**, along with my program

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Loads of thanks go to **Dr. Nicolas Costes** for his help with the french translation of my thesis abstract. Appreciation is also extended to **Dr. David Zald** (Vanderbilt University, USA) for providing the food and scenery images employed in both investigations.

The studies presented in this dissertation would not have been possible without the generous funding provided by an unrestricted research grant from **Unilever PLC (UK)** to Dr. Dagher. I am also indebted to **McGill University** and the **Graduate Program Neuroscience** for awarding me an entrance scholarship.

Many thanks go to all of the students and colleagues who crossed my path over the years and significantly impacted my life. In particular, I would like to acknowledge **Isabelle Boileau**, **Arnaud Charil**, **Hélène Hakyemez** and **Beth Tannenbaum**, former or current members of the Dagher laboratory, for making the research environment pleasurable. I would like to thank **Alex Soliman**, a recent PhD graduate from the group, for guidance in thesis writing and especially, for all of the computer-related assistance. Alex is the first person I met upon entering the program and she has become a very good friend. Also, the last few

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A huge thank you is extended to my best friends, **Angie** and **Sandy**. They undoubtedly made the PhD years memorable with all the laughter-filled good times!

Very importantly, I would like to recognize my family: **Mom**, **Dad**, **Nadia** and **Shan**. My parents have always been a positive force in my education and I would like to thank them for their constant support and encouragement; but maybe even more importantly, for the many hot meals (Mom) and transport (Dad)! Their contribution to my life really cannot be put into words and I will forever be appreciative. I would like to thank my sister for consistently reminding me that there is an exciting world beyond work, one containing *haute cuisine* and *haute couture*! I would also like to acknowledge and thank my brother for his help over the graduate years.

Finally, immense gratitude goes to my **Study Participants**. Their willingness to engage in my research endeavors has advanced the scientific knowledge of feeding behavior, thereby facilitating the completion of my doctoral degree.

ABSTRACT

Feeding behavior is a complex phenomenon involving homeostatic signals, and non-homeostatic inputs such as visual cues. In primates, exposure to food-related sensory cues has been shown to elicit cephalic phase responses as well as trigger central appetitive processing, in a motivationally-dependent manner. Neural structures consistently implicated in such responses and/or in the regulation of ingestive behavior in general, in both monkeys and in humans, include the amygdala, insula, striatum, hypothalamus, and frontal and occipital cortices. In humans however, the cerebral response to visual food stimulation remains minimally explored.

Functional Magnetic Resonance Imaging (fMRI) provides information about state-dependent changes in local neuronal activity in vivo. Using fMRI, the present dissertation examined changes in human brain activity to food and non-food pictures following the pharmacological induction of hunger with the orexigenic hormone ghrelin (Study 1), and following manipulation of the cognitive state of food expectation (Study 2).

Our data reinforce the involvement of a distributed frontal-limbic-paralimbic circuit in the central processing of food imagery, under both experimental conditions. The first study revealed that intravenous ghrelin

administration potently modulated food-associated neural responses in areas involved in reward, motivation, memory, and attention (amygdala, insula, orbitofrontal cortex, striatum, hippocampus, midbrain, visual areas). This suggests that metabolic signals such as ghrelin may promote food consumption by enhancing the appetitive response to food cues via engagement of the hedonic network.

The second study revealed that brain regions activated in the 'expectant' state (i.e. when subjects were anticipating food reward) were at least partially dissociable from those in the 'not expectant' state. In particular, recruitment of the dorsolateral prefrontal cortex, a principal component in the cognitive control network, exclusively in the 'not expectant' condition, may signal an attempt to suppress appetite in the absence of food expectation. Areas of convergence were observed in the amygdala and insula.

Obesity is rapidly becoming the major cause of excess mortality worldwide; therefore, understanding how the central nervous system controls appetite and nutrient consumption is of considerable interest. The projects in this thesis offer significant insights regarding the effects two select factors (one intrinsic and the other extrinsic) on the neural reaction to visual food stimuli, in healthy male participants.

RÉSUMÉ

Le comportement alimentaire est un phénomène complexe répondant à des signaux homéostatiques mais également des entrées non-homéostatiques, comme des signaux visuels. Chez les primates, il a été montré que l'exposition à un signal alimentaire visuel produisait des réponses céphaliques phasiques et déclanche un processus central appétitif dépendant de la motivation. Les structures neuronales spécifiquement impliquées dans une telle réponse sont l'amygdale, l'insula, le striatum, l'hypothalamus, et les cortex frontaux et occipitaux. Ce circuit est plus généralement impliqué dans le système de régulation de la prise alimentaire chez le singe et l'homme. Cependant chez l'homme, l'observation des réponses cérébrales à la stimulation visuelle induite par la nourriture reste un domaine très peu exploré.

L'imagerie par résonance magnétique fonctionnelle (IRMf) fournit des informations sur les changements d'état de l'activité neuronales *in vivo*. Cette thèse examine en IRMf les modifications de l'activité cérébrale produite par la vision d'images alimentaire et non-alimentaire, dans des conditions de faim induite pharmacologiquement par la ghréline, hormone orexigènique (Etude 1), et dans des conditions de manipulation de l'état cognitif d'attente de nourriture (Etude 2).

Nos données confirment l'implication d'un circuit fronto-limbique et paralimbique dans les processus centraux de l'imagerie alimentaire, dans les deux expériences. La première étude révèle que l'administration intraveineuse de ghréline module la réponse neuronale associée à l'alimentation dans les aires corticales impliquées dans la récompense, la motivation, la mémoire et l'attention (amygdale, insula, cortex orbito-frontal, striatum, hippocampes, cerveau médian et aires visuelles). Ces résultats suggèrent que les signaux métaboliques comme la ghréline induisent la prise alimentaire en renforçant la réponse appétitive aux signaux visuels d'aliments par l'engagement du réseau hédonique.

La deuxième étude montre que les régions cérébrales activées par l'état d'attente (i.e lorsque les sujets anticipent la récompense alimentaire) sont au moins partiellement dissociées de celles impliquées en absence d'état d'attente. En particulier, l'engagement du cortex préfrontal dorso-latéral, un des principaux composant du réseau de contrôle cognitif, dans la condition d'absence d'attente de récompense alimentaire pourrait signaler une suppression de l'appétit. On observe que les aires communes aux deux conditions restent l'amygdale et l'insula.

L'obésité est rapidement devenue une cause de mortalité excessive dans le monde; de fait, il est devenu essentiel de comprendre comment le système nerveux central contrôle l'appétit et la prise alimentaire. Le travail présenté dans cette thèse fournit une avancée significative concernant les effets de deux facteurs

sélectifs (un intrinsèque et l'autre extrinsèque) sur la réponse neuronale aux stimuli alimentaires visuels chez l'homme sain.

MODULE I:

Literature Review

PREFACE MODULE I

Module I represents the literature review section. Comprising three chapters, it summarizes the imperative background research relevant to the projects presented in this dissertation. Chapter 1 differentiates between the concepts of homeostatic and extra (or non)-homeostatic feeding, pinpointing the principal brain regions associated with these processes. Links between substrates or signals typically deemed 'homeostatic' and those deemed 'extra-homeostatic' are also covered. Chapter 2 follows with an in-depth discussion of ghrelin, a predominantly gut-produced hormone that targets neural circuitry to stimulate hunger and food intake. Finally, the last chapter (Chapter 3) provides an introduction to brain imaging. Technical features of Blood Oxygen Level Dependent (BOLD) functional Magnetic Resonance Imaging and documented research utilizing this approach to study various dimensions of ingestive behavior, are presented.

1

FEEDING

1.0 Introduction

Feeding is undeniably essential for survival. In humans however, it is also a powerful source of satisfaction and pleasure. Scientists have traditionally categorized this complex behavior into two kinds: homeostatic and extra (or non)-homeostatic, and each kind has an associated set of brain regions. In addition, current evidence suggests that interactions between so-called 'homeostatic' and 'extra (or non)-homeostatic' systems influence food consumption and energy balance.

1.1 Homeostatic Feeding Regulation

Homeostatic feeding is driven by metabolic need, and the brain is equipped with specialized circuitry that functions to ensure adequate nutrition. In this regard, the neural circuit extending from the hypothalamus to the brainstem (Figure 1-1), which is responsive to multiple metabolic signals, is considered the 'homeostatic control system' for the regulation of nutrient intake and energy balance. Attributes of this regulatory system are well-described in the literature, and are overviewed below (Hetherington and Ranson, 1942; Anand and Brobeck, 1951; Swanson and Mogenson, 1981; Woods et al., 1998; McMinn et al., 2000; Schwartz et al., 2000; Grill and Kaplan, 2002; Hillebrand et al., 2002; Saper et al., 2002; Berthoud, 2003; Schwartz et al., 2003; Travagli et al., 2003; Berthoud, 2004a, b; de Graaf et al., 2004; Broberger, 2005; Lam et al., 2005; Morton et al., 2005; Abizaid et al., 2006a; Berthoud, 2006; Gibson, 2006; Morton et al., 2006; Cameron and Doucet, 2007; Klok et al., 2007; Zheng and Berthoud, 2007; Berthoud and Morrison, 2008; Cohen and Farley, 2008; Valassi et al., 2008).

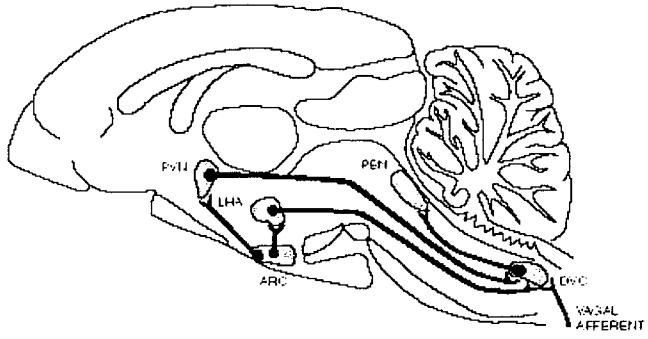


Figure 1-1. Saggital view of the integrative pathways in the hypothalamus and brainstem. PVN, paraventricular nucleus; LHA, lateral hypothalamic area; ARC, arcuate nucleus; PBN, parabrachial nucleus; DVC, dorsal vagal complex (McMinn et al., 2000).

The fundamental role of hypothalamic networks in the homeostatic regulation of feeding emerged in the first half of the 20th century. Animal studies demonstrated that lesions of the ventral medial part of the hypothalamus produced hyperphagia, while lesions of the lateral hypothalamus produced aphagia. In contrast, electrical stimulation of these regions suppressed and elicited feeding, respectively. Based on these observations, it was believed that motivated behavior is exclusively governed by these two central feeding centers ('Dual Center Model'). Even though it has since been realized that homeostatic feeding is mediated by additional structures in the brain (e.g. brainstem), the contribution of hypothalamic nuclei, especially the arcuate nucleus, to energy balance remains well supported. The importance of the arcuate stems from (among other things) the responsiveness of its neurons to diverse hormonal (and nutrient) signals relevant to feeding and energy homeostasis, such as ghrelin and leptin. Indeed, receptors selective for each of these (and other) hormones are expressed on arcuate cells, and activation of a specific receptor triggers distinct signaling cascades that result in alterations in nutrient intake. In addition to the arcuate nucleus, other hypothalamic nuclei involved in food intake and energy balance include the dorsomedial nucleus, paraventricular nucleus, ventromedial nucleus and lateral hypothalamic area. Key peptide systems implicated in the central control of feeding are harbored in these regions.

Mechanistically, the hypothalamus implements appropriate autonomic, behavioral and endocrine responses through the receipt and integration of

metabolic information originating in the trunk. This information is provided by energy status signals; namely, adiposity markers, gut hormones and nutrient-related inputs, which target this homeostatic site. To elaborate, *adiposity negative feedback signals* supply the brain with information regarding the amount and distribution of adipose tissue mass. Alterations in circulating concentrations of adiposity molecules prompt the central nervous system (CNS) to modulate nutrient intake to stabilize body fat stores. To be classified as an adiposity negative feedback signal, multiple criteria must be satisfied; presently, only leptin and insulin meet these qualifications. *Gastrointestinal peptides*, such as peptide tyrosine tyrosine (PYY), cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and ghrelin, offer information relating to the quality and quantity of nutrients consumed. While PYY, GLP-1 and CCK are post-ingestively secreted satiety hormones, ghrelin is a potent pre-prandial appetite-stimulant favoring nutrient consumption. Finally, several *nutrient-related signals* that impact the hypothalamic sites have been identified, and are believed to participate in homeostatic feeding. One example of such inputs would be free fatty acids. Activation of the hypothalamic circuitry by these various signals is mediated through either direct or indirect routes; although preliminary evidence suggests that the hormones ghrelin and PYY operate via both vagal afferents and humoral pathways to modulate food intake.

In the arcuate nucleus, two antagonistic populations of peptidergic nerve cells are the primary integrators of metabolic information. These are the

neuropeptide Y and agouti-related protein (NPY/AgRP) appetite-inducing neurons, and the proopiomelanocortin/ cocaine- and amphetamine-regulated transcript (POMC/CART) appetite-suppressing neurons. The former secrete orexigenic peptides and stimulate food intake, while the latter secrete anorexigenic peptides and inhibit feeding. Both pools of first order neurons are differentially sensitive to nutritional signals from the periphery, and it is the balance of activity between these inter-linked, yet neurochemically distinct neuronal groups that regulates consummatory behavior.

Like the hypothalamus, the brainstem's role in homeostatic feeding regulation is securing support. Information from the digestive tract is collected and transported by the vagus nerve to the brainstem nucleus of the solitary tract, where it is received and integrated. Indeed, vagal afferents synapse onto and stimulate neurons in the nucleus of the solitary tract. Moreover, the brainstem participates in feeding control via reciprocal connections with the hypothalamus.

Many of the downstream neural substrates engaged by the homeostatic centers have also been identified. Via parallel pathways, both neurons of the nucleus of the solitary tract, and also of the arcuate project further into the brain to recruit higher order brain regions. For instance, both the orexic (NPY/AGRP) and anorexic (POMC/CART) cells in the arcuate nucleus fan to neighboring hypothalamic areas specifically, the paraventricular nucleus and the lateral hypothalamus. These sites are linked with the control of nutrient intake and

autonomic function, and each produces an assortment of peptides associated with metabolic regulation. In effect, multiple brain areas are engaged into ingestive behavior and they jointly guide the nervous system to satisfy energy challenges.

In a nutshell, homeostatic feeding regulation concentrates on peripheral 'bottom-up' feeding-related signals and their actions on hypothalamic and brainstem nodes.

1.2 Non-Homeostatic Feeding Regulation

1.2.1 Overview

Substantial evidence suggests that ingestive behavior is mediated by both homeostatic and *extra (or non)-homeostatic* mechanisms. An intact metabolic system is most certainly required for the maintenance of normal energy balance, as clinical populations with genetic defects in the homeostatic system exhibit pronounced deficits in feeding regulation (e.g. Prader Willi Syndrome patients, leptin deficient patients) (Swaab et al., 1995; Farooqi and O'Rahilly, 2006). Concurrently however, factors other than intrinsic homeostatic signals drive food intake. *Extrinsic* factors, such as food cues and palatability, also play an important role in the stimulation of ingestive behavior. Extra (or non)-homeostatic feeding therefore appears to be mediated by brain regions which process the appetitive and rewarding aspects of food intake as well as emotional, cognitive and environmental inputs. Among these interconnected neural regions are the cortico-striatal loops, midbrain (VTA/substantia nigra), insula,

orbitofrontal cortex, hippocampus and amygdala. The 'reward system', represented by many of the aforementioned areas, is of particular relevance in the extra-homeostatic or hedonic control of feeding. In fact, evidence suggests that this system can surpass the hypothalamus and brainstem into an ingestive mode, even in a metabolically-replete, satiated condition (Berridge and Robinson, 1998; Saper et al., 2002; Wise, 2002; Kringelbach, 2004; O'Doherty, 2004; Broberger, 2005; Erlanson-Albertsson, 2005; Zheng and Berthoud, 2007; Berthoud and Morrison, 2008).

1.2.2 Non-Homeostatic Influences

Several non-homeostatic (or extrinsic) factors modulate food consumption. These factors are of ***hedonic*** (e.g. palatability, pleasure, food reward, reinforcing value), ***cognitive*** (e.g. expectation, dietary restraint, learned cues, eating in the absence of hunger, memorial representations of food experience) and ***environmental*** origin. Initiation and/or cessation of food intake driven by these signals appears to be somewhat distinct from basic metabolic processes and predominantly operates via an integrated ensemble of cortical, limbic and paralimbic circuits (Berridge, 1996; de Castro, 2000; Berthoud, 2003, 2004a, b; Broberger, 2005; Erlanson-Albertsson, 2005; Berthoud, 2006; Cameron and Doucet, 2007; Finlayson et al., 2007; Zheng and Berthoud, 2007; Berthoud and Morrison, 2008; Cohen and Farley, 2008).

Environmental factors are perhaps one of the most potent non-homeostatic/extrinsic factors mediating ingestive behavior. To elaborate, environmental influences such as food-related cues, food variety and food availability can have profound effects on nutrient intake and body weight. For instance, in rats, research has shown that simply increasing the availability of food strongly modifies feeding behavior (Tordoff, 2002). Specifically, animals provided five bottles of 32% sucrose solution and one bottle of water consumed appreciably more calories than did animals given five bottles of water and one bottle of 32% sucrose solution, and this was accompanied by a significant increase in body weight in the multi-sucrose bottle group (Tordoff, 2002). Additionally, primate work has revealed that the sight and visual features of food stimuli impact both the motivation to eat and region-specific neural activity (Rolls, 1994; Watanabe, 1996). Consistent with the animal literature, the importance of visual cues in humans is emphasized by the fact that more chocolate was consumed by office personnel when the candy was placed in transparent as compared to opaque containers (Wansink et al., 2006). Further, functional MRI and PET studies have demonstrated that food-related visual and olfactory cues elicit activation of numerous structures within the reward network (i.e. structures considered mesocorticolimbic, motivational or related to addiction) (comprehensive discussion in Chapter 3) (LaBar et al., 2001; Arana et al., 2003; Hinton et al., 2004; Wang et al., 2004b; St-Onge et al., 2005; Uher et al., 2006). Moreover, activation of the non-homeostatic system to such stimuli is modulated by several other inputs including, the perceived reward value of the food stimuli

(Killgore et al., 2003; Hinton et al., 2004). These findings underscore the importance of extrinsic influences as well as the sensitivity of the reward system to them.

1.2.3 Reward and Dopamine

Significant research demonstrates that the reward network participates in the modulation of the rewarding and reinforcing properties of both addictive drugs and natural reinforcers such as food. The rewarding effects of food generated by this circuit are guided by at least three neurochemical messenger systems namely, serotonin, endogenous opioids and dopamine. A brief overview of reward and dopamine is presented in the following sections.

1.2.3.1 Reward

A. The Reward System

Great strides have been made in understanding the neural substrates that sub-serve reward and motivation. In this regard, a group of inter-connected brain regions is referred to as the 'reward' system because it is implicated in the rewarding and reinforcing effects of addictive drugs and intracranial self-stimulation (Olds and Olds, 1963; Wise, 1996a, b). The mesolimbic dopamine pathway, comprising dopaminergic midbrain ventral tegmental area (VTA) neurons and their projections to the nucleus accumbens and other forebrain sites, is centrally involved in motivation and goal-directed behavior (Kelley and Berridge, 2002; Nestler, 2005). However, more recent evidence implicates

additional neural areas including the amygdala, hippocampus, hypothalamus (lateral) and prefrontal cortex (especially the orbitofrontal cortex), in the mediation of reward (Hyman and Malenka, 2001; Nestler, 2001; Saper et al., 2002; Everitt et al., 2003; Robinson and Berridge, 2003; Volkow et al., 2004; Kringelbach, 2005). These typically cognitive, affective and/or homeostatic regions interact with the nucleus accumbens and VTA reward circuits.

B. Food and Drug Reward

Data suggest that the brain reward systems are sensitive to both addictive drugs and natural rewards such as food. It has long been documented that drugs of abuse act on the mesolimbic circuit to produce reward by enhancing dopamine signaling from the VTA to the nucleus accumbens (Kelley and Berridge, 2002; Saper et al., 2002; Nestler, 2005). In other words, valuation of reward entails the secretion of dopamine from dopaminergic neurons originating in the VTA. Likewise, experimental research has proposed that the VTA-nucleus accumbens substrate governs responses to food reward (Hernandez and Hoebel, 1988a, b; Richardson and Gratton, 1996; Bassareo and Di Chiara, 1999; Kelley, 2004; Fallon et al., 2007). For instance, increases in extracellular dopamine in the nucleus accumbens, as measured by microdialysis, were observed when animals pressed a lever for food reward (Hernandez and Hoebel, 1988b), and also in freely feeding animals (Hernandez and Hoebel, 1988a). As well, feeding of a novel palatable food to ad libitum fed rats phasically stimulated *in vivo* dopamine

transmission in the medial nucleus accumbens (Bassareo and Di Chiara, 1997). The OFC, amygdala, and hippocampus have also been demonstrated to mediate various aspects of food and drug reward in both humans and animals (Breiter et al., 1997; Breiter and Rosen, 1999; Gottfried et al., 2003; Volkow et al., 2004; Fallon et al., 2007; Murray and Izquierdo, 2007; Volkow et al., 2007). Collectively, these findings support the common substrate hypothesis for natural and drug reward (Wise et al., 1978; Kelley and Berridge, 2002; Nestler, 2005). As well, it has been suggested that the brain representation of reward overlaps with neural regions catering to the sensory perception of food (Small, 2002).

Evidence suggests that chronic overindulgence of palatable food and drug addiction share common features (Berridge, 1996; Erlanson-Albertsson, 2005). The behavior generated by engaging the reward circulation is to **"come back for more"** (also known as reinforcement) (Kelley et al., 2002; Del Parigi et al., 2003) hence, the availability of appetizing foods may result in meal-related overeating due to a blunted satiety response. In fact, it has been proposed that obesity is a consequence of addiction to food (Volkow and Wise, 2005; Volkow and O'Brien, 2007). Another parallel between drugs of abuse and palatable food is that they both induce adaptations resulting in a shift in homeostatic set points. For instance, excessive food consumption revises the intrinsic set point for body weight and energy balance (Levine et al., 2003). It follows that analogous

abnormalities have been observed in brain mapping scans of natural and drug addictions (Wang et al., 2004a; Volkow et al., 2008).

C. The Dorsal Striatum

Aside from the forebrain limbic regions, one structure that is now gaining recognition as a processor of food reward is the dorsal striatum. In particular, cells that respond to food and drink reward stimuli in a fashion comparable to accumbal neurons have been identified in particular regions of the dorsal striatum, and lesions or dopamine receptor inhibitors at these sites disrupt feeding responses (Kelley and Berridge, 2002). Further, neurochemical transmission in the caudate-putamen appears to be integral to normal ingestive behavior as both standard feeding, and preference for sucrose or a palatable diet, were reinstated in hypophagic dopamine-deficient mice upon restitution of dopamine signaling in the dorsal striatum (Szczypka et al., 2001). Ventral striatal dopamine replacement merely rescued preference for hedonic feeding (Szczypka et al., 2001). Dopamine flow in the caudate-putamen appears to also be implicated in food reward-related learning (Beninger and Ranaldi, 1993). Finally, human brain imaging studies favor a role for the dorsal striatum in food motivation and feeding that is dissociable from its role in mediating reward through the nucleus accumbens (Volkow et al., 2002; Small et al., 2003).

D. Liking versus Wanting

Food is said to be rewarding when it elicits positive emotions that increase the motivational drive to acquire it. Kent Berridge has proposed that food reward can be parsed into the psychological components of *liking* and *wanting*. These processes appear to be separable, and are directed by distinct central substrates and neurochemical systems (Berridge, 1996).

Liking (pleasure/palatability) refers to the hedonic impact of a food stimulus. The typical (favorable) facial expressions generated by humans and animals when experiencing a pleasant taste has been described as the most basic manifestation of the liking phenomenon. Nutrition-related liking is primarily directed by opioid neurotransmission and is mediated by anatomical regions such as the ventral pallidum, nucleus accumbens shell and brainstem (Berridge, 1996; Berridge and Robinson, 2003).

Wanting (or incentive salience) is the second element of reward. Representation of this process within the context of food reward involves parts of the nucleus accumbens and amygdala, and the (mesolimbic) dopamine system (Berridge, 1996; Berridge and Robinson, 2003). While experimental manipulation of mesolimbic pathways vigorously affects wanting (i.e. instrumental performance for and consumption of food), it does not modulate liking (Berridge, 2003; Berridge and Robinson, 2003; Cannon and Palmiter, 2003; Pecina et al., 2003). Of interest, the wanting circuitry appears to be closely

associated with components of the homeostatic CNS feeding system, hence metabolic hormones can modulate *wanting* for food via direct effects on mesolimbic dopaminergic cells (Berthoud and Morrison, 2008).

E. Modulation of Food Reward

Human neuroimaging studies suggest that two dissociable mechanisms modulate food reward, possibly via distinct neural substrates (Small, 2002). First, changes in the incentive value of foods have been observed on the basis of energy state (i.e. hunger versus satiety) (Small et al., 2001) and second, changes in reward value have been observed on the basis of sensory-specific satiety (i.e. reductions in the reward value of foods eaten to satiety but not of other novel foods) (O'Doherty et al., 2000). Some evidence indicates that the former may be mediated by the insula and the latter by the orbitofrontal cortex.

1.2.3.2 Dopamine

Dopamine is a catecholamine neurotransmitter that has been implicated in ingestive behavior. Four dopaminergic pathways; namely, tuberoinfundibular, nigrostriatal, mesolimbic and mesocortical, participate in feeding behavior (Tataranni and DelParigi, 2003) and five receptor subtypes facilitate dopamine's actions (Blum et al., 2000). Changes in dopaminergic neurotransmission mediated predominantly by dopamine receptor subtype 2, appears to be the leading mechanism by which dopamine directs food intake and reward (Wang et al., 2001; Wang et al., 2004a). Further, animal experiments have shown that

ingestion mobilizes dopamine in the reward network, particularly in the nucleus accumbens (Hernandez and Hoebel, 1988a, b; Radhakishun et al., 1988; Yoshida et al., 1992; Westerink et al., 1994; Richardson and Gratton, 1996; Bassareo and Di Chiara, 1997, 1999), and local injections of dopamine into the ventral striatum has itself been demonstrated to trigger nutrient consumption (Swanson et al., 1997). As well, mesolimbic dopamine activity appears to be related to the rewarding properties of foods (Martel and Fantino, 1996) and the dopaminergic circuitry is now a recognized target for feeding-associated peptides such as ghrelin and leptin (Abizaid et al., 2006b; Fulton et al., 2006) .

The long held belief that dopamine encodes hedonic value (Wise, 1985) has been challenged (Berridge and Robinson, 1998; Schultz, 1998). For instance, negative interference with dopamine activity (e.g. neurotoxic 6-hydroxydopamine lesions or dopamine antagonists) fails to abolish the pleasurable (i.e. hedonic or 'liking') responses to sweet taste reward (Berridge, 1996; Berridge and Robinson, 1998). Moreover, while the dopamine response is enhanced when rewards are first introduced, it gradually habituates even though animals continue to seek the reward thereafter (discussed in (Saper et al., 2002)). As well, with repeated pairings in which a particular cue reliably signals reward, dopaminergic responses in primates are shifted from reward receipt to the conditioned cues that anticipate and predict future rewards (Schultz, 1998, 2002). These developments therefore favor a role of dopamine in reward-related learning (Schultz, 1998) and/or incentive salience ('wanting') (Berridge, 2007) rather than hedonics. Indeed, the

possibility that dopamine encodes more general functions such as sensorimotor integration, arousal and/or attention has also been proposed (Grigson, 2002; Kelley and Berridge, 2002).

Overall, the complex relationship between dopaminergic systems and reward has yet to be fully understood. Nevertheless, it would appear that dopamine modulates reward circuitry and its necessity in feeding is stressed by the observation that (genetically) dopamine-deficient mice die of starvation (Zhou and Palmiter, 1995).

1.2.4 Regions of Interest

In the following sections, the insula, orbitofrontal cortex, and amygdala, three crucial appetitive structures associated with pathways tagged as 'extra-homeostatic', are individually addressed. It is important to note that although feeding-related brain regions are often functionally labeled as 'homeostatic' or 'extra-homeostatic', certain areas may participate in both the 'homeostatic' and 'extra-homeostatic' control of energy balance.

1.2.4.1 Insula

The insula, a paralimbic structure positioned within the lateral sulcus, contains the primary gustatory cortex, and mediates different dimensions of nutrient-related processing (Augustine, 1996; Scott and Plata-Salaman, 1999). Human neuroimaging studies display activation of this region to taste stimuli

(O'Doherty et al., 2001; Del Parigi et al., 2002a; De Araujo and Rolls, 2004; Kringelbach et al., 2004), to food odors (O'Doherty et al., 2000) as well as to food pictures (LaBar et al., 2001; Killgore et al., 2003; Wang et al., 2004b; Simmons et al., 2005; St-Onge et al., 2005). These findings are consistent with the proposed role of the insula in the integration and analysis of multi-modal inputs (Augustine, 1996). Additionally, this region has been implicated in the 'imagination' of favorite foods and food craving (Pelchat et al., 2004), thirst (Denton et al., 1999) and in the processing of reward (Small et al., 2001). While hunger-induced insular activity has been observed in humans (Tataranni et al., 1999; Morris and Dolan, 2001; Small et al., 2001; Porubska et al., 2006; Cornier et al., 2007), monkey research fails to show modulation of neuronal firing rate based on energy status (i.e. by feeding to satiety) (Rolls et al., 1988; Yaxley et al., 1988). Ergo, the primate data suggest that the insula represents taste (identity and intensity) independent of reward (Rolls et al., 1988). Primate work has additionally shown that the primary taste cortex is located in the *anterior* insula and, that this area houses neurons encoding sweet, bitter, salt, sour and umami tastes as well as neurons tuned for somatosensory characteristics of foods (e.g. viscosity, fat, texture) (Scott et al., 1986; Yaxley et al., 1990; Baylis and Rolls, 1991; Rolls et al., 1996; Verhagen et al., 2004). Evidence also supports a role for the insula in interoception (i.e. the sense of the physiological condition of the body) (Craig, 2002).

Anatomically, the insular cortex is coupled with several regions in the central nervous system. In both animals and humans, this area has connections with the cerebral cortex (e.g. orbitofrontal cortex), basal ganglia, other limbic regions (e.g. entorhinal cortex, hippocampus), and the dorsal thalamus. These associations likely facilitate the insula's feeding-specific activities (Yasui et al., 1991; Augustine, 1996; Yamamoto, 2006).

1.2.4.2 Orbitofrontal Cortex

The orbitofrontal cortex (OFC) is a large and heterogeneous structure located on the ventral surface of the frontal lobe. It represents the prefrontal area that receives projections from the mediodorsal nucleus of the thalamus. Much progress has been made regarding the functions and neuroanatomical organization of the OFC (reviewed in Kringelbach and Rolls, 2004; Rolls, 2004; Kringelbach, 2005; Rolls, 2005; Barbas, 2007; Murray and Izquierdo, 2007; Murray et al., 2007; Price, 2007; Rempel-Clower, 2007; Small et al., 2007).

Research in non-human primates has significantly shaped current thinking regarding the OFC and its role in feeding behavior. Neurophysiological studies have demonstrated that distinct neurons in this region respond to the sight, smell, and taste of food, and that food-associated activity in these cells is modulated by motivational state (Rolls et al., 1989; Rolls et al., 1990; Critchley and Rolls, 1996; Rolls et al., 1999; Rolls, 2000; Rolls et al., 2003). In other words, the firing rate of OFC neurons to food-related stimuli is enhanced when monkeys are hungry

and diminished after satiation. Further, 'sensory-specific satiety' is also observed (i.e. decreases in neural activity to a food on which the animal has been fed to satiety, while neuronal responses to other foods are maintained) (Rolls et al., 1989; Critchley and Rolls, 1996; Rolls et al., 1999; Rolls, 2000; Rolls et al., 2003). OFC neurons therefore appear to represent the current reward value or motivational significance of food stimuli (Critchley and Rolls, 1996; Rolls et al., 1999; Rolls, 2004).

Confirmation for the primate OFC's function in certain facets of reward assessment is provided by lesion studies. OFC damage impairs the ability to alter behavioral responses following food-reward devaluation (Izquierdo et al., 2004; Machado and Bachevalier, 2007; Murray and Izquierdo, 2007). However, such lesions fail to disrupt preferences for palatable foods (Machado and Bachevalier, 2007). These findings imply that the OFC is not only involved in hedonics, but also in the control over behavior in relation to appetite or motivation.

Consistent with the primate literature, human neuroimaging studies corroborate a role for the OFC in encoding reward value (O'Doherty et al., 2000; Gottfried et al., 2003; Hinton et al., 2004). A functional MRI investigation by O'Doherty and colleagues, showed that brain activation of an area of the OFC selectively decreased to a banana odor yet not to a control vanilla odor after bananas were consumed to satiety (O'Doherty et al., 2000). A similar pattern of OFC activity was also observed in a parallel study using food-related predictive

visual cues, instead of odors (Gottfried et al., 2003). These findings suggest that the OFC tracks changes in reward value such that OFC responsivity is exclusively reduced to (conditioned) cues associated with the devalued food. Notably, reward-related activation in this region has additionally been observed using other unique approaches paired with brain mapping techniques (Arana et al., 2003; Hinton et al., 2004) (further discussed in Chapter 3).

Two interesting trends regarding the human OFC's representation of reward have surfaced from a meta-analysis of 87 imaging studies exhibiting trustworthy OFC activation (Kringelbach and Rolls, 2004). First, it would seem that the OFC's representation of reward is localized to distinct sub-regions depending on the type of reinforcer. More specifically, taste and odor rewards are encoded more posteriorly while abstract rewards such as money, more anteriorly. Second, the medial and lateral OFC may have separate roles whereby the former is involved in monitoring the reward value of reinforcers while the latter is involved in assessing punishers (Kringelbach and Rolls, 2004).

Insights regarding the OFC's contribution to other aspects of appetitive processing have also been provided by human brain imaging studies. For instance, signal in the OFC is modulated by food-related visual, olfactory and taste stimuli (O'Doherty et al., 2001; Small et al., 2001; Gottfried et al., 2003; Hinton et al., 2004; Simmons et al., 2005). Further, anterior OFC activity has been shown to co-vary with recognition memory score for both food and non-food

pictures, suggesting that the OFC mediates memory operations (Morris and Dolan, 2001). Indeed, several brain mapping investigations suggest the OFC (and likely the amygdala) possess integral roles in the acquisition, storage and recall of representations of experiences with food (Berthoud, 2007; Zheng and Berthoud, 2008). The OFC has also been implicated in denoting the subjective pleasantness of a food stimulus (Kringelbach et al., 2003), in flavor representation (de Araujo et al., 2003; Small et al., 2007) as well as in both the expectation, and receipt of a taste reward (positive and negative valence) (O'Doherty et al., 2001; O'Doherty et al., 2002). Indeed, the OFC (caudolateral portion) has been referred to as a secondary taste cortical area (Rolls et al., 1990; Baylis et al., 1995).

From an anatomical perspective, the OFC has numerous connections. In particular, it receives inputs from multiple regions including the insula, amygdala, thalamus, striatum, cingulate cortex, hypothalamus, hippocampus and other parts of the prefrontal cortex, and most input connections are reciprocal. Projections of the OFC to the striatum and prefrontal areas may educe (feeding-related) behavioral responses while projections of this region (and also of the amygdala) to the hypothalamus may mediate appropriate autonomic and endocrine responses linked to food processing (Kringelbach and Rolls, 2004; Rolls, 2004; Kringelbach, 2005; Barbas, 2007; Murray and Izquierdo, 2007; Murray et al., 2007; Price, 2007; Rempel-Clower, 2007).

A striking feature of the OFC is that it receives numerous sensory inputs. In particular, the OFC receives inputs from olfactory, gustatory, somatosensory and visual association cortex. With respect to the latter, animal studies demonstrate that visual information transmitted through visual cortical areas (V1, V2 and V4) reaches the inferior temporal cortex and is then predominantly routed to lateral areas of the OFC. Taken together, the abundance of sensory inputs suggest that the orbital network is likely involved in the appraisal of food and food-associated stimuli (Kringelbach and Rolls, 2004; Rolls, 2004; Kringelbach, 2005; Barbas, 2007; Murray and Izquierdo, 2007; Murray et al., 2007; Price, 2007; Rempel-Clower, 2007). Kringelbach (2005) suggests that because the OFC receives vast inputs from different sensory modalities, it may be a critical locale for representing incentive salience and hedonic experience (Kringelbach, 2005).

1.2.4.3 Amygdala

The amygdala is an anatomically complex and multi-functional structure embedded deep within the medial temporal lobe. It is composed of several nuclei. Although the relatively low spatial resolution of functional Magnetic Resonance Imaging limit human amygdala findings to the whole region, animal research shows that each component nucleus has unique inputs, outputs and functions. In general, the amygdala has been associated with an array of activities such as emotion, reward, memory, learning and attention. Some of these functions are mediated in collaboration with one or more of the following regions, the prefrontal cortex, striatum, hippocampus and hypothalamus, with which the

amygdala has connections (Swanson and Petrovich, 1998; Baxter and Murray, 2002; Cardinal et al., 2002; Sah et al., 2003; Phelps, 2004; LeDoux, 2007; Murray, 2007; Murray and Izquierdo, 2007; Talarovicova et al., 2007).

Recent findings broaden the recognized roles of the amygdala to the appetitive sphere. Human brain mapping studies have reported amygdala activation to visual food stimuli (LaBar et al., 2001; Morris and Dolan, 2001; Killgore et al., 2003; Hinton et al., 2004; Führer et al., 2008), thereby supporting primate work which has identified local amygdala neurons that are selectively responsive to the sight of food (Nishijo et al., 1988; Ono et al., 1989; Rolls, 1994). Further, amygdala activity to food cues appears to be modulated by energy status such that there is enhanced activity following food restriction (LaBar et al., 2001; Morris and Dolan, 2001; Führer et al., 2008). This suggests that the amygdala may be involved in the integration of representations of biologically salient rewards with current metabolic state. Indeed, aside from visual stimuli, imaging experiments also exhibit amygdala activation to food-related gustatory and olfactory stimuli (O'Doherty et al., 2000; O'Doherty et al., 2001; Smeets et al., 2006).

In addition, some feeding-related amygdala functions are carried out in concert with the OFC as these areas are robustly and reciprocally connected. Among these are taste, flavor and reward processing (O'Doherty et al., 2001; Baxter and Murray, 2002; Gottfried et al., 2003; Hinton et al., 2004; Izquierdo

and Murray, 2004; Izquierdo et al., 2005; Murray, 2007; Small et al., 2007), and reward expectation (O'Doherty et al., 2002). Given the intimate relationship between the amygdala and OFC, and their role in reward, it is not surprising that combined lesions of the two regions (both cross-hemispheric and unilateral) impair performance in the Reinforcer Devaluation paradigm (Baxter et al., 2000; Murray and Izquierdo, 2007). Notably, reward-related activities mediated by the amygdala entail communication with other cortical and sub-cortical reward substrates including the nucleus accumbens, ventral tegmental area, substantia nigra and prefrontal cortex (Baxter and Murray, 2002; Murray, 2007) as well. Last, like the OFC, the amygdala also represents both positive and negative affective valence (O'Doherty et al., 2001) and directs learning and memory (Morris and Dolan, 2001; Cardinal et al., 2002; McGaugh, 2004; LaBar and Cabeza, 2006; Murray and Izquierdo, 2007). With respect to the latter, in humans, amygdala responses have been found to co-vary positively with recognition memory for food items (Morris and Dolan, 2001).

1.3 Interactions Between the Feeding Systems

Research advances provide persuasive evidence for an *interaction* between entities considered 'homeostatic' and those considered 'extra (or non)-homeostatic'. A number of circulating metabolic signals traditionally associated with the homeostatic circuitry have now been revealed to additionally converge on the reward system in both humans and animals. For instance, human brain imaging studies have reported that the intravenous administration of leptin and

PYY, two peripheral hormones that suppress food intake, altered activity in the insula, striatum and/or OFC as well as modulated appetitive behavioral responses (further discussed in Chapter 3) (Baicy et al., 2007; Batterham et al., 2007; Farooqi et al., 2007). Similarly, rodent experiments have demonstrated that the orexigenic hormone ghrelin activates neurons in the VTA, increases dopamine turnover in the nucleus accumbens and potently induces food intake when locally administered in the VTA (Naleid et al., 2005; Abizaid et al., 2006b; Jerlhag et al., 2007). Taken together, the aforementioned observations suggest that metabolic hormones may influence feeding behavior via modulation of cortico-meso-limbic reward processing. Indeed, these findings are reinforced by the expression of specific receptors for ghrelin, leptin and PYY in reward-mediating brain regions, in addition to their presence in the homeostatic structures (Ohkubo et al., 1990; Guan et al., 1997; Burguera et al., 2000; Berthoud, 2004b; Zigman et al., 2006).

Furthermore, it has been proposed that the nucleus accumbens and the hypothalamus may be the key integrative sites linking homeostatic and non-homeostatic feeding programs. On the one hand, outputs fanning from the hypothalamus to the nucleus accumbens may influence the motivation to eat via molecular signals. On the other hand, outflow from the ventral striatum to the hypothalamus (especially the lateral hypothalamic area) may facilitate the ability of mesolimbic processes, stimulated by relevant hedonic and environmental cues, to stimulate the metabolic networks, and enhance energy intake. In particular, it has been postulated that the nucleus accumbens can disinhibit the orexigenic

neurons in the lateral hypothalamic region thereby engaging the hypothalamic peptidergic systems typically involved in homeostatic appetite control (Kalivas and Nakamura, 1999; Stratford and Kelley, 1999; Swanson, 2000; Zahm, 2000; Berthoud, 2004a, 2006, 2007).

It must be stated however that other reward processing areas such as the amygdala, ventral tegmental area and frontal cortex, also provide inputs into the hypothalamus, which may additionally contribute to the interactions between the two feeding systems (Berthoud, 2007).

1.4 SUMMARY

Researchers in the field of ingestive behavior have separated feeding into two types, each of which is linked to a specific set of brain regions. The first type, *homeostatic feeding*, is based on nutrient deficiency and operates to normalize body weight. It is mediated by CNS areas which sense and interpret intrinsic metabolic signals (e.g hormones) and neural signals, and in turn produce adaptive feeding-related responses, namely, the hypothalamus and brainstem. Figure 1-2 provides a pictorial depiction of the hierarchical organization of homeostatic feeding regulation.

The second type, *extra-homeostatic feeding*, occurs independently of physiological need and is greatly driven by extrinsic signals (e.g. food cues and palatability). Comparative research has revealed that this kind of feeding is

facilitated by a group of cortical, limbic and paralimbic structures. These substrates process cognitive, emotional and environmental factors/inputs as well as the rewarding features of feeding. The reward system, consisting of areas such as the VTA, striatum, hippocampus, amygdala and OFC, is particularly important in the extra-homeostatic or hedonic control of food intake as it imparts hedonic/reward tags to a variety of behaviors, including ingestion. Two integral structures involved in reward processing or more specifically, in the efficient analysis of food rewards (or associated stimuli) are the orbitofrontal cortex and amygdala. Notably however, these areas have also been implicated in other aspects of feeding behavior.

Additionally, interactions between feeding systems defined as 'homeostatic' and 'extra-homeostatic' are evident. For instance, signaling molecules that normally guide metabolic mechanisms have been shown to target the hedonic circuitry to modulate ingestive behavior. Further, anatomical links between the hypothalamus and mesolimbic areas indicate the existence of circuits that facilitate cross-talk between the homeostatic feeding centers and reward substrates. In effect, these circuits may not only allow basic communication, but perhaps even overpower homeostatic control by facilitating the dominance of feeding in states of positive energy supply. Figure 1-3 illustrates the multidimensional aspects of energy balance.

Figure 1-2. The central metabolic circuitry is regulated by numerous endocrine and neural inputs. Schematic illustration of how brain networks regulating ingestive behavior communicate with peripheral organs.

Abbreviations: Arc: arcuate nucleus of the hypothalamus; DMX: dorsal motor nucleus of the vagus; IML: intermediolateral cell column; NPY: neuropeptide Y; nTS: nucleus of the solitary tract (brainstem); OEA: oleoylethanolamide (lipid mediator); POMC: proopiomelanocortin; PYY: peptide YY (Broberger, 2005).

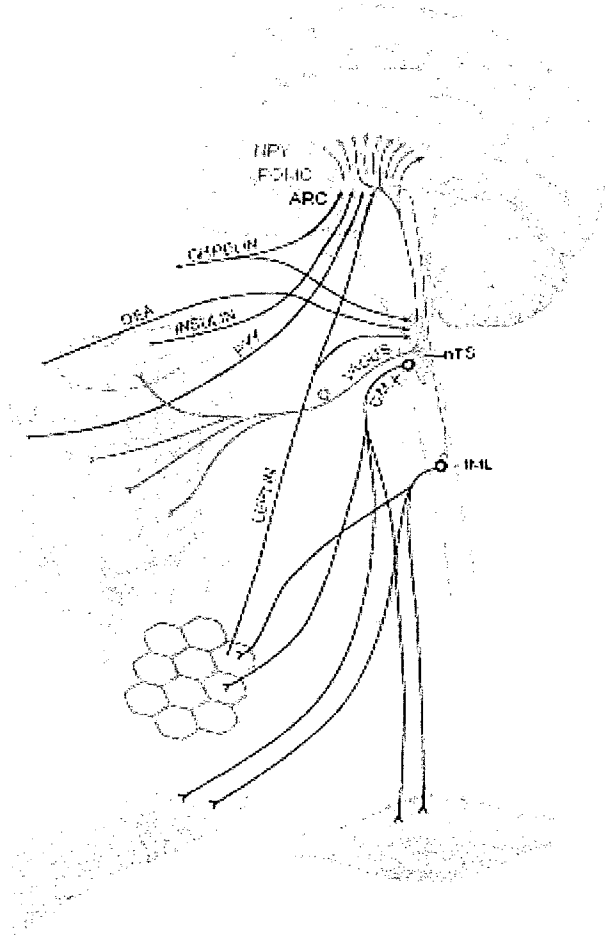
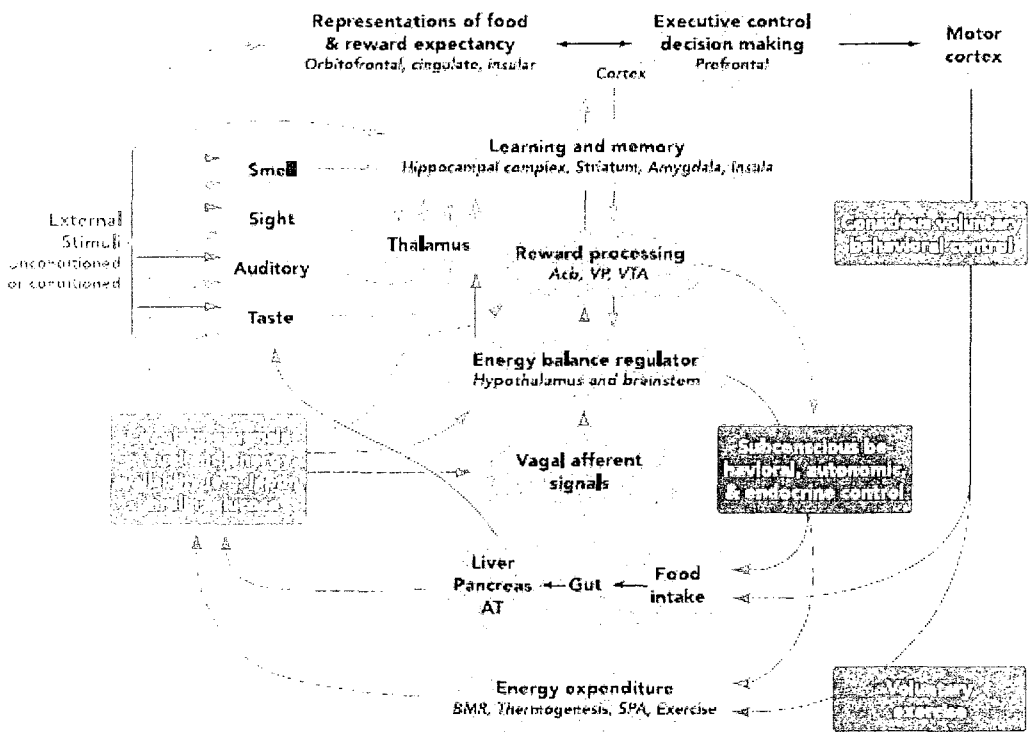


Figure 1-3. Major systems and pathways responsible for the neural integration of internal and external information in the control of appetite and energy expenditure.

Blue areas and pathways are mainly involved in metabolic and energy balance regulation. Red areas and pathways are mainly involved in communication with the external world through cognitive and emotional processes such as learning and memory, reward, mood, stress, choice and decision making (Zheng and Berthoud, 2008).



2

GHRELIN

2.0 Introduction

Considerable research has shown that the hypothalamic peptidergic circuits play a fundamental role in the control of appetite and metabolism (Chapter 1). The isolation of *ghrelin* by Kojima and colleagues has added another dimension to the hypothalamic regulation of energy balance. Ghrelin, a gut-generated hormone, is a potent appetite stimulant that targets the central homeostatic circuitry. However, ghrelin binding in areas such as the hippocampus, ventral tegmental area, substantia nigra and raphe nuclei, suggest that this orexigenic peptide may additionally participate in the rewarding and motivational aspects of ingestive behavior, as well as in higher brain functions such as learning and memory.

2.1 Structure and Forms

Ghrelin is a 28 amino acid peptide with an n-octanoylated serine 3 residue, that was isolated from rat stomach less than a decade ago (Kojima et al., 1999). This structure is unique in mammalian physiology and likely mediates ghrelin's bioactivity (Kojima et al., 1999). Specifically, post-translational acylation, catalyzed by the enzyme GOAT (ghrelin o-acyltransferase) (Yang et al., 2008), is believed to augment ghrelin's lipophilic properties, perhaps even facilitating transport across the blood brain barrier. Interestingly, human ghrelin differs from rat ghrelin by only two amino acids (Kojima et al., 1999; Hosoda et al., 2003), and is present in human blood at substantial plasma concentrations (Kojima et al., 1999). The structural sequence of the human peptide is the following:

H-Gly-Ser-Ser(octanoyl)-Phe-Leu-Ser-Pro-Glu-His-Gln-Arg-Val-Gln-Gln-Arg-Lys-Glu-Ser-Lys-Lys-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg-OH

Two major forms of ghrelin have been identified in tissues and plasma, n-octanoyl-modified ghrelin and des-acyl (non-acylated) ghrelin (Hosoda et al., 2000a, b; Hosoda et al., 2003). In humans, a 117 amino acid ghrelin precursor (preproghrelin), with a secretory signal sequence, is cleaved and chemically-processed to yield the 28 amino acid peptide (Kojima et al., 1999; Hosoda et al., 2003), which is the major active form of ghrelin. However, alternative splicing of the precursor yields several minor forms of the peptide, including des-acyl ghrelin (Hosoda et al., 2003). Des-acyl ghrelin may be a pre-form of ghrelin and/or a

product of its degradation, as 'active' ghrelin is relatively unstable (Hosoda et al., 2004). Functionally, non-acylated ghrelin appears to lack ghrelin's endocrine and metabolic effects (Kojima et al., 2001; Broglio et al., 2003a; Neary et al., 2006). All subsequent reference to ghrelin will imply the octanoylated, bioactive form.

2.2 Sources

The principal site of ghrelin production in vertebrates is the stomach (Ariyasu et al., 2001). Ghrelin-containing cells, known as X/A-like cells, contain round, compact, electron-dense granules that are filled with ghrelin, and are one of four endocrine cell types in the oxyntic mucosa of both rodents and humans (Date et al., 2000). In the adult human, ghrelin cells represent ~20% of the endocrine cell population of oxyntic glands in the fundus (Date et al., 2000). Notably, in humans, total gastrectomy has been reported to significantly reduce plasma ghrelin levels (to roughly 50% of those pre-gastrectomy) when measured 30 minutes post-operatively; however, over a few months, ghrelin concentration gradually increases to approximately 70% of the original. This suggests that while the bulk of ghrelin is derived from the stomach, resection of the structure may result in compensatory production by extra-gastric sites (see below) (Hosoda et al., 2003).

In addition to gastric ghrelin, lower amounts of the peptide are obtained from other sources. These include peripheral organs such as the intestines, pancreas, kidney, placenta, gonads, lungs and eye, plus various neoplastic tissues

and cancer cell lines (reviewed in (Hosoda et al., 2000b; Kojima et al., 2001; Kojima and Kangawa, 2005; Sato et al., 2005; Hosoda et al., 2006; Leite-Moreira and Soares, 2007)).

Finally, mounting data have indicated production of the peptide in the brain. Particularly, ghrelin has been identified in the pituitary and in the hypothalamic nuclei (including arcuate, dorsomedial, ventromedial and lateral) as well as in formerly uncharacterized hypothalamic neurons adjacent to the third ventricle between the dorsal, ventral, paraventricular and arcuate hypothalamic nuclei (Kojima et al., 1999; Hosoda et al., 2000b; Korbonits et al., 2001b; Korbonits et al., 2001a; Lu et al., 2002; Cowley et al., 2003; Sato et al., 2005).

2.3 Functions

In terms of function, ghrelin was originally identified as a potent growth hormone-releasing agent (Kojima et al., 1999). Acting through specific receptors that are densely concentrated in the hypothalamus, growth hormone secretagogues (GHS) have long been known to potently trigger growth hormone (GH) release (Ghigo et al., 1997; Smith et al., 1997; Deghenghi, 1998; Arvat et al., 2000). Ghrelin is an endogenous ligand for the GHS receptor (re-named 'ghrelin receptor') through which it strongly stimulates GH secretion in animals (Kojima et al., 1999; Seoane et al., 2000), in humans (Arvat et al., 2000; Peino et al., 2000; Takaya et al., 2000; Hataya et al., 2001) and in vitro (Kojima et al., 1999).

Apart from its growth hormone releasing capability, the peptide has several other physiological activities. These include *gastric functions* (Masuda et al., 2000; Date et al., 2001; Dornonville de la Cour et al., 2004), *cardiovascular functions* (Nagaya et al., 2001a; Nagaya et al., 2001b; Nagaya et al., 2001c; Pettersson et al., 2002; Nagaya and Kangawa, 2003), *sleep-related functions* (Weikel et al., 2003; Dzaja et al., 2004; Szentirmai et al., 2006; Kluge et al., 2008), *other hormone release* (Takaya et al., 2000; Arvat et al., 2001; Muccioli et al., 2002; Arosio et al., 2003; Broglio et al., 2003c; Schmid et al., 2005), and *feeding / energy balance functions* (Tschop et al., 2000; Asakawa et al., 2001; Nakazato et al., 2001; Wren et al., 2001a; Wren et al., 2001b). Specific effects of ghrelin are listed in Table 2-1.

Table 2-1. Effects of Ghrelin.

Hormonal Effects	
Growth hormone release	↑
Adrenocorticotrophic hormone release	↑
Cortisol release	↑
Prolactin release	↑
Thyroid stimulating hormone release	?
Luteinzing hormone release	?
Follicle-stimulating hormone release	?
Insulin release	?
Anabolic Effects	
Appetite	↑
Adiposity	↑
Cardiovascular Effects	
Cardiac output	↑
Blood pressure	↓
Apoptosis of cardiomyocytes in vitro	↓
Gastric Effects	
Gastric acid secretion	↑
Gastric motility	↑
Sleep-Related Effects	
Slow-wave sleep	↑

↑= increase, ↓= decease, ?= variable data (Table slightly modified from (Kojima and Kangawa, 2005, 2006))

2.4 Receptors

The ghrelin receptor (GHS-R) is a 7 transmembrane pass G-protein coupled receptor which exists in at least 2 forms: GHS-R1a and GHS-R1b (Howard et al., 1996). The latter is produced by an alternative splicing mechanism and appears to be pharmacologically inactive (Howard et al., 1996);

hence, GHS-R1a is thought to mediate ghrelin's biological activities. Recently, it has been shown that GHS-R1a exhibits high constitutive activity in vitro (~50% activity) (Holst et al., 2003).

The distribution pattern of ghrelin receptors is quite extensive and favors the host of proposed functions. In the central nervous system, ghrelin receptors (or ghrelin receptor mRNA) have been located in the pituitary, hippocampus, substantia nigra, ventral tegmental area, dorsal median raphe nuclei, all 3 components of the dorsal vagal complex (area postrema, nucleus of the solitary tract and dosal motor nucleus of the vagus), facial motor nucleus, cerebellar cortex and multiple hypothalamic nuclei (e.g. arcuate, paraventricular and ventromedial) (Howard et al., 1996; Bennett et al., 1997; Guan et al., 1997; McKee et al., 1997; Mitchell et al., 2001; Gnanapavan et al., 2002; Cowley et al., 2003; Zigman et al., 2006). Ghrelin receptors have also been identified in the vagal nodose ganglion (Date et al., 2002b). Finally, many peripheral organs including, the heart, spleen, pancreas, adrenal gland and thyroid express GHS-R (Guan et al., 1997; Gnanapavan et al., 2002).

2.5 Clinical Pharmacokinetics

Human pharmacokinetic data is available from two studies. A 2001 phase I clinical trial involving the intravenous injection of a single 10 μg/kg ghrelin bolus revealed that plasma ghrelin levels increased to 61-times the baseline value approximately one minute after ghrelin injection and the peptide was eliminated

with a half-life of about 10 minutes. This investigation additionally reported favorable hemodynamic effects (i.e. increased cardiac output, decreased mean arterial pressure and no change in heart rate) following ghrelin administration (Nagaya et al., 2001a). However, a lack of randomization and a small sample size (n=6 male subjects) challenged the validity of the results (Figure 2-1).

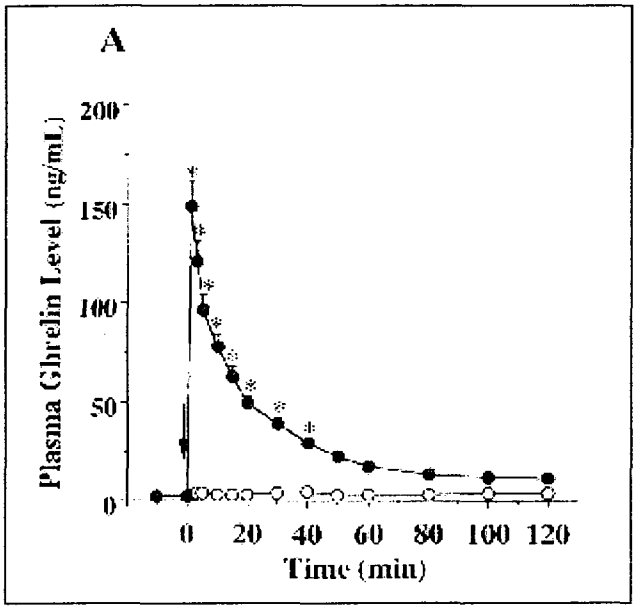
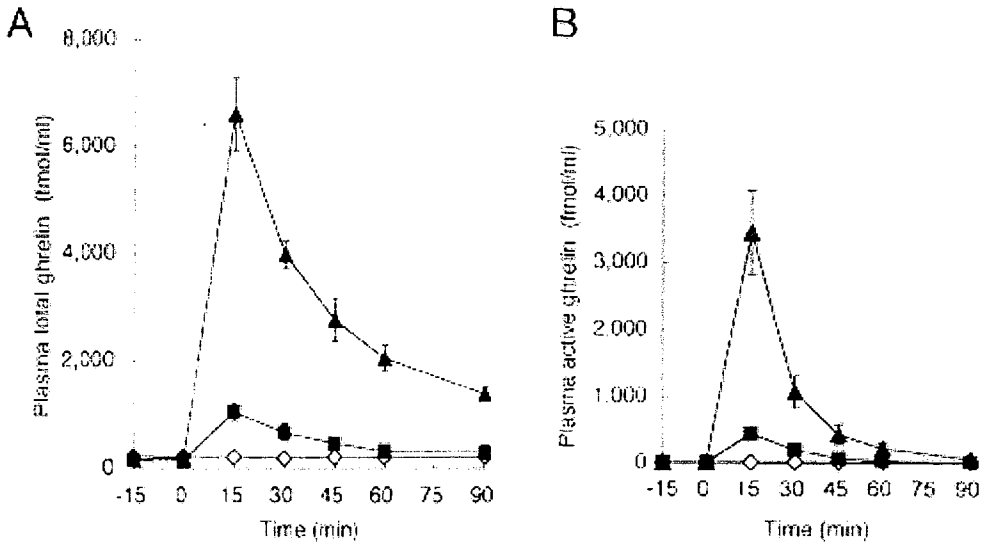


Figure 2-1. Circulating ghrelin levels after a single injection of ghrelin (10 μg/kg). Data are means ± SEM. *P<0.05 vs. placebo group. The arrow indicates an intravenous injection of ghrelin or placebo (Nagaya et al., 2001a).

More recently, a randomized, double-blind and placebo-controlled trial with 18 subjects re-examined the pharmacokinetics and safety of ghrelin, as well as addressed its efficacy in the induction of appetite and hormone secretion (Akamizu et al., 2004). Participants received one of 3 intravenous treatments: low dose ghrelin (1 μg/kg), high dose ghrelin (5 μg/kg) or placebo (3.75% D-Mannitol). A number of side effects to ghrelin administration were reported including, abdominal discomfort, somnolence, flushing, glucosuria, hyperhidrosis; yet, they were short-lived and well-tolerated. The total plasma ghrelin

concentration (acylated and des-acylated) 15 minutes post-injection of both ghrelin doses (1 and 5 μg/kg), was comparable to that observed in the aforementioned Nagaya et al study, in which a dose of 10 μg/kg was administered (Nagaya et al., 2001a). Acylated ghrelin constituted 42-52% of the total ghrelin concentration at the 15-minute post-injection mark, and had an elimination half-life of 9-13 minutes, indicating that it is rapidly cleared from the circulation, while total ghrelin's half-life was three times longer, averaging 27-31 minutes. This investigation additionally confirmed ghrelin's ability to markedly trigger growth hormone release, and stimulate hunger (Akamizu et al., 2004).

Figure 2-2. Pharmacokinetics of intravenous bolus administration of ghrelin. Plasma ghrelin concentrations were measured by C- and N-RIA for total (A) and active ghrelin (B) respectively. ▲, 5μg/kg ghrelin; ■, 1μg/kg ghrelin; □, placebo. All values are expressed as means ± 95 % CI (n = 6 (A) and n = 5 (B)). (Akamizu et al., 2004)



2.6 Feeding, Memory and Reward

2.6.1 Feeding

2.6.1.1 Studies in Animals

A. Overview

Ghrelin's ability to instigate feeding and augment body weight in animals is well described (Kamegai et al., 2000; Tschop et al., 2000; Wren et al., 2000; Kamegai et al., 2001; Nakazato et al., 2001; Shintani et al., 2001; Wren et al., 2001b; Lawrence et al., 2002). In 2000, Tschop et al were the first to demonstrate that *systemic* administration of ghrelin (for 14 days) produces weight gain by decreasing fat use, and continuous *central* administration (for 7 days) elicits a dose-dependent increase in hyperphagia and body mass (Tschop et al., 2000). In addition, ghrelin has been shown to potently enhance food consumption when delivered through various routes (intracerebroventricular, intraperitoneal, subcutaneous, central site-specific) (Tschop et al., 2000; Wren et al., 2000; Wren et al., 2001b), and recurrent administration of the peptide fails to noticeably attenuate the ingestive response (Wren et al., 2001b). Ghrelin's role in feeding is further validated by the observation that administration of anti-ghrelin immunoglobulin G significantly suppresses food intake in both fasted (8 hours) and ad-libitum fed rats (Nakazato et al., 2001). Importantly, the induction of feeding by this peptide is independent of its growth hormone stimulatory activity (Kamegai et al., 2000; Tschop et al., 2000; Nakazato et al., 2001; Shintani et al., 2001).

B. The Hypothalamus

Several important observations link ghrelin's feeding-related functions to the hypothalamus, a region of primary importance in the regulation of feeding. First, it has been shown that systemic or intracerebroventricular administration of ghrelin activates multiple hypothalamic nuclei pertinent to feeding including the arcuate, paraventricular and dorsomedial nuclei as well as the lateral hypothalamic area (Hewson and Dickson, 2000; Lawrence et al., 2002; Scott et al., 2007; Kobelt et al., 2008). Second, local injections of ghrelin into hypothalamic sub-regions significantly triggered energy consumption, especially when the arcuate nucleus was targeted (Wren et al., 2001b). Third, both peripherally and centrally administered ghrelin have been demonstrated to up-regulate gene expression of neuropeptide Y (NPY) and agouti gene-related protein (AGRP), two potent orexigenic peptide transmitters that co-reside in a population of arcuate nucleus neurons (Hewson and Dickson, 2000; Kamegai et al., 2000; Asakawa et al., 2001; Kamegai et al., 2001; Nakazato et al., 2001; Shintani et al., 2001), and this was accompanied by an elevation in food intake (Kamegai et al., 2000, 2001). Fourth, ghrelin neuron axons are directly associated with NPY/AGRP orexigenic neurons in the hypothalamic arcuate nucleus (Cowley et al., 2003). Last, antagonism of either NPY or AGRP neural signaling diminished the appetite-stimulating response of injected ghrelin (Asakawa et al., 2001; Nakazato et al., 2001; Shintani et al., 2001).

Together, the data suggest that ghrelin targets the NPY/AGRP hypothalamic neurons, 94% of which express ghrelin receptors (Willesen et al., 1999), to stimulate the production and secretion of NPY and AGRP, resulting in an increase in feeding.

C. Feeding and Other Neural Regions

Ghrelin appears to modulate appetitive responses via structures other than the hypothalamus. Direct application of ghrelin into the hippocampus, ventral tegmental area, nucleus accumbens, dorsal raphe nucleus and brainstem, have all been shown to potently trigger food consumption, often in a dose-related manner (Faulconbridge et al., 2003; Carlini et al., 2004; Naleid et al., 2005; Abizaid et al., 2006b). Moreover, with respect to the brainstem, at least two sites, the nucleus of the solitary tract and the area postrema, are activated by exogenous ghrelin admnistration (Lawrence et al., 2002). It is quite possible that other central areas are also implicated in ghrelin-mediated energy balance as evidenced by the widespread distribution of ghrelin receptors (section 2.4).

D. Link to Leptin

Leptin and ghrelin appear to have opposite effects in nutrient regulation (Klok et al., 2007). The findings that central ghrelin administration reversed both leptin-induced feeding inhibition as well as the reduction in hypothalamic NPY mRNA expression (Nakazato et al., 2001; Shintani et al., 2001) suggest that there is a competitive interaction between ghrelin and leptin in feeding regulation. In

other words, ghrelin seems to offset leptin's well-recognized anorexigenic effects thereby signaling hunger and stimulating feeding. These antagonistic actions of leptin and ghrelin, are at least in part mediated by hypothalamic neuronal populations as they express receptors selective for each of the two peptides (Hakansson et al., 1996; Mercer et al., 1996; Fei et al., 1997; Guan et al., 1997; Elmquist et al., 1998; Burguera et al., 2000; Cowley et al., 2003; Zigman et al., 2006)

E. Meal Initiation

Ghrelin's postulated role in pre-meal hunger and meal initiation is extensively described in a recent review article (Cummings, 2006). Though the present discussion is not exhaustive, a few *general* findings supporting this function include the following. First, administration of exogenous ghrelin has been shown to effectively stimulate ingestive episodes in ad libitum fed (sated) animals and the peptide's effects are fast and brief (Tschop et al., 2000; Wren et al., 2000; Nakazato et al., 2001; Lawrence et al., 2002). Next, ghrelin injections appear to modulate the appetitive phase of feeding (e.g. elevations in meal initiation episodes, foraging, hoarding, etc) rather than the consummatory phase (Keen-Rhinehart and Bartness, 2005). Last, exogenous ghrelin increases the number of meals (via a decrease in the latency to feed) rather than meal size (Cummings, 2006).

In addition to the above indirect observations, more *direct* experimental evidence also hints that preprandial ghrelin is a hunger signal. Two studies examining the diurnal rhythm of the peptide in sheep, have shown that animals habituated to feeding either once, twice or four times per day, exhibited surges in ghrelin levels prior to each meal, whereas freely-feeding animals showed only small (insignificant) fluctuations in ghrelin concentrations throughout the day (Sugino et al., 2002a; Sugino et al., 2002b). Such pre-meal peaks have also been observed in other species (Miura et al., 2004; Drazen et al., 2006). Further, research has indicated that endogenous ghrelin and/or its gastric mRNA levels are elevated by fasting, and lowered by re-feeding or oral glucose administration (Tschop et al., 2000; Murakami et al., 2002). Importantly, distension of the stomach with water administration failed to decrease endogenous ghrelin concentrations (Tschop et al., 2000; Williams et al., 2003). Rather nutrient-induced suppression of circulating ghrelin appears to be the result of a pre- or post-absorptive response such as changes in intestinal osmolarity or insulin concentrations (Williams et al., 2003). Collectively, these findings implicate peptide levels in meal initiation and food consumption. Indeed, preprandial increases in ghrelin may be a consequence of (or signal) food expectation in animals (Sugino et al., 2002a; Sugino et al., 2002b).

F. Mechanism of Action

Ghrelin's capacity to induce food intake requires activation of the neural circuitry. While centrally produced ghrelin is accessible to hypothalamic and

other local ghrelin receptors, peripherally secreted ghrelin must also target these binding sites. Data have shown that gastric ghrelin can penetrate the blood brain barrier but at a variable rate (Banks et al., 2002); thus, it seems likely that peripheral ghrelin stimulates orexigenic regions via an indirect pathway as well. The presence of ghrelin receptors on vagal afferent neurons in the rat nodose ganglion imply that ghrelin signals from the gut are transmitted to the brain via the vagus nerve (Date et al., 2002a; Zigman et al., 2006). In addition, intracerebroventricularly injected ghrelin has been found to stimulate c-fos, a marker of neuronal activation, in the neurons of the nucleus of the solitary tract and the dorsomotor nucleus of the vagus, further displaying activation of the vagus system (Date et al., 2001). In contrast, vagotomy suppresses ghrelin's appetite-inducing effects (Date et al., 2002a; le Roux et al., 2005b). Importantly however, the vagus nerve's contribution to ghrelin signaling is contradictory. Opposite to the above, a recent study reports that the acute appetite-stimulatory effect of ghrelin is independent of vagal signaling (Arnold et al., 2006). Until further clarification, it is assumed that peripheral ghrelin modulates central orexigenic circuitry via both direct and indirect routes.

2.6.1.2 Studies in Humans

The relationship between ghrelin and nutritional homeostasis in man is only beginning to be recognized. In fact, the original experiments investigating the effects of exogenous ghrelin administration in humans predominantly focused on the peptide's endocrine activities. Interestingly, in the majority of these

investigations, a common and intense side effect reported by participants was the perception of hunger (Arvat et al., 2000; Arvat et al., 2001; Broglio et al., 2001; Aimaretti et al., 2002; Broglio et al., 2002a; Broglio et al., 2002b; Di Vito et al., 2002; Broglio et al., 2003c; Broglio et al., 2003b). This observation sparked massive research exploring ghrelin's contribution to human feeding behavior.

The majority of information advocating ghrelin's role in appetite and energy balance has come into view from endogenous ghrelin studies. Consistent with the animal data, human research suggests that intrinsic ghrelin levels trigger feeding episodes. More specifically, plasma levels were found to nearly double before each meal, and fell to trough levels within an hour after food was consumed (Cummings et al., 2001). Furthermore, elevation in ghrelin levels consistently occurred in subjects voluntarily initiating meals without food or time related cues (thereby dissociating preprandial increases from an anticipation effect), and there was a temporal overlap between ghrelin levels and hunger scores (Cummings et al., 2004). Notably, post-prandial decreases in peptide concentrations seem to be proportional to the caloric content of the meal (Callahan et al., 2004; le Roux et al., 2005a). Collectively, these findings demonstrate a link between human ghrelin levels and the acute (i.e. short-term) regulation of energy balance; particularly, implicating the hormone as a physiological meal initiator.

A comprehensive understanding of normal physiology often requires evidence from clinical states. Circulating ghrelin levels were discovered to be decreased in human obesity (Rigamonti et al., 2002; Tschop et al., 2001) as well as following gastric bypass surgery (Cummings et al., 2002). The exception to the former, are Prader-Willi patients who have severe hyperphagia and extremely and constantly high ghrelin levels, indicating a dysfunction in the central satiety system (Cummings et al., 2002; Hinton et al., 2006). In contrast, upregulation of ghrelin concentrations has been observed in anorexia patients (Rigamonti et al., 2002). Notably, ghrelin levels decrease when anorexia nervosa patients gain weight, and increase following dietary weight loss in obese humans (Otto et al., 2001; Cummings et al., 2002; Hansen et al., 2002). Therefore, in addition to its short-term effects on hunger and meal initiation, ghrelin appears to signal long-term changes in body weight and energy balance.

Human studies administering *exogenous* ghrelin to evaluate its role in feeding regulation are now being pursued. Wren and colleagues (2001) were the first to reveal a direct relationship between ghrelin and feeding behavior in man and have thus far, been the major group to explicitly examine the effects of ghrelin administration on appetite and food intake (Wren et al., 2001a). Employing a randomized, double-blind, experimental design, they described a statistically significant increase in energy consumed by all subjects from a buffet meal during *intravenous* ghrelin (5pmol/kg/min) relative to saline infusion. Visual analog scores for appetite were also greater during ghrelin treatment.

Subsequent studies replicated these effects in healthy subjects following *subcutaneous* administration of ghrelin (Druce et al., 2006), and also in obese subjects receiving intravenous ghrelin (Druce et al., 2005). The latter implies that sensitivity to ghrelin is retained in obesity. Worth mentioning, exogenous ghrelin does not appear to stimulate food intake in patients with surgical procedures involving vagotomy (le Roux et al., 2005b). In addition to its effects on food intake, this hormone appears to incite the imagination of food. More precisely, subjects reported **"vivid plastic images"** of their preferred meal following receipt of exogenous ghrelin (Schmid et al., 2005). Last, ghrelin infusion has been shown to increase the gastric emptying rate in normal weight humans (Levin et al., 2006).

2.6.2 Memory

Behavioral and morphology experiments in rodents suggest that ghrelin affects memory processes (Carlini et al., 2002; Carlini et al., 2004; Diano et al., 2006). To elaborate, non-specific intracerebroventricular (ICV) administration of the peptide to rats, dose-dependently increased the latency time in the step-down test of inhibitory avoidance when given post-training, indicating memory retention (Carlini et al., 2002). Ghrelin delivered directly into the hippocampus, amygdala and dorsal raphe nucleus, reproduced the aforementioned results in behavioral memory testing thereby localizing ghrelin's influences on memory operations to distinct central structures (Carlini et al., 2004). Further, even systemic ghrelin has been discovered to modulate cognitive behavior and

hippocampal morphology (Diano et al., 2006). Specifically, in addition to enhancing the performance of animals in a spatial memory task, peripherally-administered ghrelin was shown to reach and bind to neurons in the hippocampus, and induce spine synapse formation in the CA1 region. As well, ghrelin treatment produced hippocampal long-term potentiation (LTP) in slice preparations. Thus, Diano et al showed that synaptic alterations produced by ghrelin were paralleled by increased spatial learning and memory (Diano et al., 2006).

Ghrelin's role in learning and memory is further confirmed by the improvement of ghrelin-treated SAMP8 mice in behavioral memory testing (Diano et al., 2006). The senescence-accelerated prone mouse (SAMP8) is a genetic model used in studies of aging and Alzheimer's disease (Nomura and Okuma, 1999; Morley et al., 2000). Due to deficits in cognitive abilities and learning, these mice are progressively impaired in behavioral memory testing. In fact, as mentioned by the investigators (Diano et al., 2006), by the age of 12 months, SAMP8 animals are impaired in the T-maze footshock avoidance task. Remarkably, exogenous ghrelin administration enhanced test performance in 12 month old mice as well as in 4 month old animals, suggesting better memory (Diano et al., 2006).

Finally, ghrelin's role in the mediation of memory processes is supported by the fact that the hippocampus, a memory-related structure, is populated with ghrelin receptors (Diano et al., 2006).

2.6.3 Reward and Dopamine

Mounting research demonstrates that ghrelin targets the central dopaminergic reward system. Abizaid and colleagues showed that ghrelin augments activity of dopamine neurons in the ventral tegmental area (VTA), a crucial node in the reward circuit. They additionally reported that peripheral administration of the peptide induced synapse formation on these same cells. In vivo, systemic ghrelin delivery was demonstrated to enhance dopamine turnover in the nucleus accumbens in both wild type and ghrelin-null mice. As this response was absent in ghrelin receptor (GHSR) knockouts, it would appear that the hormone's actions are GHSR-dependent (Abizaid et al., 2006b). In another investigation, ICV administration of ghrelin elevated ventral striatal dopamine as well as induced an acute increase in locomotor activity (Jerlhag et al., 2006). The latter effects were also observed following the direct administration of ghrelin into both the VTA and the laterodorsal tegmental area (Jerlhag et al., 2007). Collectively, these findings suggest that ghrelin can activate the mesolimbic dopamine pathways originating in the VTA, and increase dopamine in target areas such as the nucleus accumbens.

Ghrelin's ability to impinge on the mesolimbic reward circuit also appears to modulate feeding, a behavior previously linked to this pathway (briefly mentioned in section 2.6.1.1 C.) (Abizaid et al., 2006b). Local injections of ghrelin into both the VTA and the nucleus accumbens elicited feeding in ad-libitum fed animals (Naleid et al., 2005; Abizaid et al., 2006b); with the response

being two-fold greater when ghrelin was administered into the VTA (Naleid et al., 2005). This is consistent with the presence of ghrelin receptors in the VTA but not in the nucleus accumbens (Zigman et al., 2006; Guan et al., 1997). Notably however, the identification of ghrelin-positive neuronal processes in the nucleus accumbens is suggestive of possible signaling in this region (Cowley et al., 2003). Further, the orexigenic effect of circulating ghrelin was suppressed by the administration of a ghrelin receptor antagonist into the VTA (Abizaid et al., 2006b). Hence, it appears that ghrelin's appetite-stimulatory effects are, at least partially governed by the VTA and the nucleus accumbens (Naleid et al., 2005; Abizaid et al., 2006b) and further, appear to be independent of opioid signaling (Naleid et al., 2005). These findings suggest that ghrelin enhances feeding by acting on the mesolimbic dopamine system, likely modulating the reward value of food.

Markedly however, not all components of the reward network are sensitive to ghrelin treatment to enhance feeding responses. For instance, energy consumption was not modified when ghrelin was administered into the amygdala (Carlini et al., 2004). While ghrelin receptors have not been identified in this region, ghrelin-containing axon terminals have been detected (Cowley et al., 2003).

Indeed, other studies also challenge, or do not endorse, ghrelin's actions on the (dopamine-gated) reward systems. For instance, Korotkova and colleagues

report that the firing rate and membrane potential of VTA neurons was not modulated by ghrelin (Korotkova et al., 2006). And, a recent publication advocates that ghrelin-mediated food consumption is driven by energy requirements rather than by reward (Bomberg et al., 2007). Briefly, in the latter investigation, ad-libitum fed rats given a choice between a calorie-dense chow versus a calorie-dilute sucrose solution, ingested more chow following both ICV and hypothalamic-paraventricular infusions of ghrelin (Bomberg et al., 2007). Since animals favored the energy dense food over the palatable sweet liquid, the authors argue that ghrelin's main action is to augment food intake to meet metabolic needs. Importantly however, the Bomberg study cannot reject the possibility that ghrelin participates in reward feeding.

Further research is necessary to clarify ghrelin's role in reward. Nonetheless, preliminary observations suggest that enrichment of reward processing in the mesolimbic dopamine network is a component of ghrelin's orexigenic effects.

2.7 Ghrelin and Imaging

At present, there exist no neuroimaging studies with ghrelin. However, very recently, three functional MRI papers examining the effects of leptin and peptide YY, two peripherally-secreted satiety signals, on CNS circuitry have been published (Baicy et al., 2007; Batterham et al., 2007; Farooqi et al., 2007). Detailed discussion of these studies is presented in Chapter 3.

2.8 Summary

Ghrelin is an acylated hormone that is implicated in a spectrum of biological activities including feeding and memory. To elaborate, ghrelin is a hot addition to the neuroendocrine network that regulates energy homeostasis. Synthesized in the gut and in the central nervous system, this orexigenic signal predominantly targets the hypothalamic (NPY/AGRP) circuitry, where specific receptors are present, to affect appetite and food consumption. However, emerging evidence indicates that ghrelin also modulates core brain circuits that process reward and motivation to influence feeding behavior. As well, roles for this hormone in both short-term and long-term ingestive control are evident.

Aside from its participation in energy balance, ghrelin influences learning and memory systems. Rodent models indicate that exogenous ghrelin treatment improves performance in memory-related behavioral testing, induces synaptic remodeling in the hippocampus and produces LTP. The presence of ghrelin binding in the hippocampus supports these findings.

Interestingly, it has recently been proposed that ghrelin may represent a molecular link between memory and metabolic control (Diano et al., 2006). Certainly, memorial representations pertaining to experiences with food contribute to normal feeding regulation.

3

NEUROIMAGING

3.0 Introduction

Functional neuroimaging is a term that incorporates a number of techniques that facilitate the mapping of the living brain. Functional Magnetic Resonance Imaging (fMRI), one such method, was developed in the 1990s (Ogawa et al., 1990) and provides an indirect assessment of neural function. Gaining recognition as a methodological tool, fMRI is increasingly being employed to examine brain activation associated with cognitive and pharmacological challenges. In particular, whole-brain blood oxygen level dependent fMRI has assisted in disclosing the neuroanatomical substrates of feeding behavior.

3.1 Blood Oxygen Level Dependent fMRI

Blood oxygen level dependent (BOLD) fMRI measures changes in the MR signal in cortical and sub-cortical structures via the coupling between blood oxygenation and blood flow. The fundamental principle underlying this technique is that mental tasks that stimulate neuronal activity augment regional cerebral blood flow (CBF). This elevation in CBF usually exceeds the level that is required to supply the cells with oxygen, resulting in an increase in the oxygenated to deoxygenated hemoglobin ratio. As deoxyhemoglobin is an intrinsic paramagnetic contrast agent, variations in MR signal are detected based on the degree of oxygenation. In effect, BOLD fMRI is a pseudo-quantitative means of inferring neuronal signaling. Relative to other imaging techniques, fMRI has many advantages. It is non-invasive, does not involve radiation or external contrast agents, and has excellent spatial resolution. Furthermore, despite the approximate 6 to 8 second delay between the neuronal event and the hemodynamic response, the temporal resolution is also relatively good. The major disadvantage of fMRI is movement artifact, either of the whole head or of the brain itself (e.g. with respiratory or cardiac cycles) (Huettel et al., 2004).

3.2 MRI Data Analysis

The image analysis process strives to identify (region-specific) voxels that exhibit changes in MR signal in the task versus control condition. Preprocessing of raw data, the initial stage, entails the *realignment* of brain volumes to minimize motion, and *spatial smoothing* to reduce noise. Thereafter, the General Linear

Model (GLM), a parametric method to assess the information at each voxel, is applied. The formula for the GLM is given by:

$$Y = a_0 + a_1x_1 + a_2x_2 + \ldots + a_nx_n + e$$

The equation implies that the observed data (y) is equal to a weighted combination of several model factors (x_i) plus an additive error term (e). Model factors encompass theorized changes in BOLD activity associated with the manipulations of the independent variables or other known sources of variability. The parameter weights (a_i) reflect the amount that each factor contributes to the overall data and the term a_0 indicates the total contribution of all factors that are held constant during the experiment. Upon estimating the parameters, T-maps can be calculated. Multiple software packages are available for fMRI analysis, including SPM, Brain Voyager and AFNI. Data analysis for this thesis was conducted with in-house generated "fMRIstat" software (Worsley et al., 2002). The fMRIstat program involves 3 basic functions. In the first step, termed *fmridesign*, information regarding the paradigm is input into the program. Thereafter, *fmrilm*, integrates this information to locate activation in a single functional run. Finally, step 3 (*multistat*) combines the statistical output of *fmrilm* within or across subjects. This sequential process yields t-statistic images, which can subsequently be thresholded using the minimum given by a Bonferroni correction and random field theory, in order to identify stimulus-related neural activity.

Various arithmetical computations can be performed with fMRIstat; namely, fixed, mixed, or random effects, analyses. A 'fixed effects' analysis examines changes in the group mean signal relative to the group within subject variance. While this method provides lots of degrees of freedom, taking into account the overall fit of the model (all parameters), it does not account for subject to subject variability. Results may thus be driven by only a few subjects, preventing the generalization of conclusions to the population as a whole. In using this approach, the probability of Type 1 error (false positive) is increased. Alternatively, a random or mixed effect model can de employed. Despite fewer degrees of freedom, such paradigms incorporate both within and between subject variances. In this case however, the possibility of Type II error (false negative) is elevated. While several options exist, standard protocol dictates the performance of a fixed effects analysis within a subject and a mixed effects analysis across subjects (Worsley et al., 2002; Huettel et al., 2004). This systematic procedure optimizes the analysis of native data.

3.3 Positron Emission Tomography

Positron Emission Tomography (PET) is another imaging method that permits the examination of neural tissue in vivo. It entails the use of radiolabeled variants of biologically significant molecules that emit signal when they decay. Different markers that trace distinct physiologic processes are available. For instance, [^{18}F]FDG (fluorodeoxyglucose) measures glucose metabolism and [^{15}O]H_2O tracks blood flow. In addition, several radiotracers that are ligands for

specific neuroreceptor subtypes, such as [^{11}C]raclopride for dopamine D2 receptors, have also been developed. The basic idea is that once a radiopharmaceutical is injected, it distributes in the tissue depending on its chemistry, and on the brain's metabolic and blood flow demands. PET is thus an indirect measure of tissue metabolism. Unlike fMRI however, it is minimally invasive, involves radioactivity and is more expensive (Grasby, 2002; Tataranni and DelParigi, 2003).

3.4 Neuroimaging and Feeding Behavior

3.4.1 fMRI and PET with Visual Food Stimuli

Functional imaging coupled with the passive viewing of real food or associated stimuli such as food pictures, has only recently been employed to examine feeding regulation in humans. Although such PET and fMRI studies are limited in number, they offer integral insights regarding the central nervous system's response to food cues (LaBar et al., 2001; Morris and Dolan, 2001; Volkow et al., 2002; Killgore et al., 2003; Wang et al., 2004b; Simmons et al., 2005; St-Onge et al., 2005; Beaver et al., 2006; Killgore and Yurgelun-Todd, 2006; Porubska et al., 2006; Uher et al., 2006; Cornier et al., 2007; Führer et al., 2008).

First, the neural response to food cues appears to be modulated by motivational state. Labar and colleagues, the first to publish using this methodology, detected amygdala, fusiform, and parahippocampal gyrus activation

to pictures of food compared to pictures of tools, exclusively in the hunger state. Since this activity did not extend to the state of satiety, the involvement of the amygdala and related inferotemporal regions in the integration of intrinsic energy status with motivationally-relevant sensory cues was proposed (LaBar et al., 2001). While this hunger-driven amygdala response to images of food has been replicated by two groups (Morris and Dolan, 2001; Führer et al., 2008), others have failed to identify activity in this region (St-Onge et al., 2005; Porubska et al., 2006; Uher et al., 2006; Cornier et al., 2007). Notably, these different experiments vary in the duration of food restriction (5 hours to 24 hours), in the sample size, and in the type and strength of scanner employed (PET versus fMRI; 1.5T versus 3.0T). In addition to the amygdala, visual food-related responses in the nutrient-depleted (hunger) state have been observed in multiple other structures; namely, the OFC, hippocampus, anterior cingulate cortex, hypothalamus, thalamus, temporal areas, dorsal striatum, cerebellum, striate/extrastriate cortex and insula (Morris and Dolan, 2001; Volkow et al., 2002; Wang et al., 2004b; St-Onge et al., 2005; Porubska et al., 2006; Uher et al., 2006; Cornier et al., 2007; Führer et al., 2008). Indeed, the degree of hunger may modulate insular and OFC activity as responses in these regions have been found to correlate with subjective ratings of appetite and hunger (Wang et al., 2004b; Porubska et al., 2006). Overall, these findings indicate that energy status has a significant effect on the central response to visual food stimuli.

Second, the neural response to food imagery is influenced by the motivational salience (caloric content) of the images. While the amygdala appears to be generally reactive to visual food stimuli regardless of energy content (Killgore et al., 2003), the perceived reward value of food engages separate prefrontal systems; with pictures of calorie-dense foods activating the medial and dorsolateral prefrontal cortex, and calorie-dilute foods activating the medial OFC (Killgore et al., 2003). Notably, the OFC has also been shown to be recruited following exposure to high calorie foods (Simmons et al., 2005). Indeed, both prefrontal systems (i.e. orbital and medial prefrontal) have been implicated in aspects of appetitive and goal-directed behavior, as well as in emotion (Killgore et al., 2003). In addition, affect modulates neural responses to high and low calorie foods (Killgore and Yurgelun-Todd, 2006, 2007). In particular, images of calorie-rich foods elicited activation of satiety-linked areas (lateral OFC) and hunger-linked areas (medial OFC and insula) during positive and negative affect, respectively; suggesting, that the aforementioned brain structures may contribute to cravings for palatable foods during adverse emotional states.

Third, there is significant personality-based variability to the brain response to highly palatable food cues. Beaver et al report that individual differences in 'reward drive' (as measured by the Behavioral Activation Scale), are positively correlated with activity in the core hedonics and reward network (i.e. ventral striatum, amygdala, midbrain, orbitofrontal cortex and ventral pallidum). The authors suggest that these findings may help explain increased

vulnerability to specific feeding disorders in certain individuals (Beaver et al., 2006).

Fourth, food picture-induced neural activity is shaped by feeding regimens. In a unique experimental design, thin, obesity resistant subjects underwent fMRI after two days of eucaloric intake, and after two days of overfeeding (30% overfeeding), in a counterbalanced design. Results showed that hedonically appealing images (compared to neutral images) yielded less activity in visual cortical areas as well as in the hypothalamus in the overfed versus the eucaloric condition, suggesting that the salience of appetizing food cues is reduced in a state of energy surplus. Furthermore, overfeeding also lessened hunger ratings, and elevated post-meal satiety ratings. The brain activation patterns along with subjective hunger/satiety scores imply that during a state of energy surplus, the gain on the metabolic system is altered, favoring restoration of normal nutritional balance.

Fifth, visual appetitive stimuli appear to modulate dopamine signaling. A [^{11}C] raclopride PET study in food-deprived humans, showed that food stimulation increased extracellular dopamine in dorsal but not ventral striatum, and that these increases correlated positively with self-reports of hunger and desire for food (Volkow et al., 2002). (This finding has been replicated in a PET/feeding study employing a visual cue-free paradigm. In particular, consumption of a preferred meal was associated with enhanced dopamine release

in the dorsal striatum, and this response correlated positively with meal pleasantness ratings (Small et al., 2003)). Hence, food reward may be mediated by dopamine in the dorsal striatum, while other rewards (eg. drugs of abuse) by dopamine in the nucleus accumbens. This supports experimental evidence in animals (Chapter 1).

Last, the cerebral response to visual food stimulation appears to be disrupted in clinical populations with eating-related disorders. Anorexia nervosa patients exhibit differential central processing of visually presented food images relative to healthy controls (Gordon et al., 2001; Santel et al., 2006). Also, compared to normal-weight subjects, obese individuals exhibit enhanced neural activity in many reward-related and associated regions (e.g. dorsal striatum, hippocampus, insula, amygdala and/or ventral striatum) to pictures of high calorie foods in both hungry (8-9 hours fasted) and neutral (i.e. in the absence of hunger or satiety) energy states (Rothemund et al., 2007; Stoeckel et al., 2008). The presence of increased reward system activation to palatable food cues in a neutral energy state suggests that it may be a factor in pathological overeating and obesity (Rothemund et al., 2007).

3.4.2 Other Feeding-Related Imaging Studies

Diverse facets of human ingestive behavior have been explored using imaging protocols void of visual food items or pictures. One early [H_2O^{15}] PET study simply examined the neuroanatomical correlates of hunger (36 hour fast)

and satiation (after liquid meal), in the absence of *any* food-related sensory stimulation. Hunger was associated with significantly increased regional CBF (indicative of brain activation) in the hypothalamus, insula, OFC, dorsal striatum (caudate and putamen), hippocampus, anterior cingulate, parahippocampal gyrus, thalamus, precuneus and cerebellum, while satiation was associated with increased regional CBF in the prefrontal cortex (ventromedial and dorsolateral) and inferior parietal lobule (Tataranni et al., 1999). From a functional perspective, the structures recruited during the hunger condition have been linked with reward, memory, attention, internal state perception, habit learning, emotion, and goal-directed behavior, and the satiety-engaged areas are implicated in inhibitory control and response suppression (Tataranni et al., 1999). Further, the diffuse structural activation associated with hunger supports an influential theory suggesting that nutrient regulation inherently favors weight gain via more pronounced responses to energy restriction than to energy surpluses (Tataranni et al., 1999; Schwartz et al., 2003; DelParigi et al., 2005a). Notably, differential neural responses to hunger and satiation have been observed between the sexes (Del Parigi et al., 2002b), and between obese versus lean individuals (Gautier et al., 2000; Gautier et al., 2001; DelParigi et al., 2005b).

Two PET studies attempted to dissect the biological substrates of internal metabolic status from external incentive factors to the motivation to eat. In a clever paradigm, investigators imaged subjects presented with restaurant menus listing preferred (high incentive) and less preferred (low incentive) meal choices

in both hungry and satiated states. The results of the experiments showed that distinct brain regions are engaged in hunger (amygdala, insula, hypothalamus, striatum, medulla, anterior cingulate cortex) and satiety (temporal cortex, lateral OFC), and apparently, satiety shifted and reduced structural activation relative to the nutrient-deprived condition. These data support the work of Tataranni and colleagues described above (Tataranni et al., 1999). Furthermore, of these motivation-associated regions, only the OFC and amygdala were responsive to the appetitive incentive value of foods items (high incentive only). These findings insinuate that neural areas mediating the mechanisms by which homeostatic (intrinsic) and extra-homeostatic (extrinsic) factors influence motivation to eat are partially separable, with intersection in the amygdala and OFC (Arana et al., 2003; Hinton et al., 2004).

Food craving is a significant determinant of nutrient intake. This intense desire to consume particular foods appears to affect obesity and/or other eating-related disorders. In a fMRI investigation evaluating the neural substrates of this phenomenon, cravings were elicited by having subjects envision the sensory properties of preferred foods and of a monotonous liquid meal, following a dietary challenge (Pelchat et al., 2004). Neuronal responses associated with craving were identified in the hippocampus, insula and caudate. It would appear that the mere thought of liked foods is sufficient to recruit components of the reward and memory networks.

Effects of gradual ingestion on functional brain activity have also been probed. In particular, chocolate, a commonly craved substance, was evaluated in a creative PET study at the Montreal Neurological Institute (Small et al., 2001). Brain scans acquired during progressive chocolate consumption revealed differential activation between when subjects were hungry and **"motivated to eat"** chocolate, and when they ate in the satiated state. Specifically, when chocolate was gratifying, the midbrain, caudomedial OFC, insula and striatum were engaged, and as it became less pleasurable, the parahippocampal gyrus, caudolateral OFC and prefrontal regions. The feeding-related (i.e. fasted to fed) switch in OFC responsivity from the caudomedial to the caudolateral site, suggests that these sub-areas of the OFC may participate in the instigation and cessation of feeding, respectively (Small et al., 2001). Further, in a separate study, a significant correlation between decreases in subjective pleasantness as a liquid food is eaten to satiety and a region of the human orbitofrontal cortex, has been reported (Kringelbach et al., 2003). Overall, modulation of activity in the OFC and insula regions in the above investigations, support a role for these structures in the representation of reward and/or in hunger/satiety-based feeding responses (Tataranni et al., 1999; Small et al., 2001; Kringelbach et al., 2003).

A unique means of assessing central representations of reward value is the sensory specific satiety paradigm. Briefly described in Chapter 1, sensory specific satiety is defined as ***"a form of reinforcer devaluation in which participants that have been fed to satiety on one food cue will still find other***

foods rewarding, and will eat some of these foods" (Kringelbach, 2005). Functional MRI studies based on this concept report that as a food odor or a visual predictive cue associated with a food is devalued (by having subjects eat the associated food to satiety), BOLD signal in the amygdala and/or OFC is accordingly reduced. However, neural activity in these regions is maintained when cues or odors for the non-devalued food items are presented (O'Doherty et al., 2000; Gottfried et al., 2003). Hence, it is speculated that these areas monitor changes in reward value (O'Doherty et al., 2000; Gottfried et al., 2003), thereby supporting primate research (Rolls et al., 1989; Critchley and Rolls, 1996; Baxter et al., 2000; Rolls, 2000).

To conclude, although a few key gustatory and olfactory studies have been referenced in this section, a thorough discussion of the imaging data with respect to these sensory modalities is beyond the scope of this thesis. Suffice to say, some of the brain regions responsive to visual food cues, are also responsive to food-related taste and/or smell stimuli.

3.4.3 Imaging Expectation

Expectation to eat is a cognitive factor that influences ingestive behavior (Berridge, 1996). At present, only one imaging study has examined the role of expectancy in feeding. O'Doherty and colleagues (2002) compared the neuroanatomical substrates implicated in the *anticipation* of a taste reward to those involved in *receipt* of reward, using fMRI. During the task, participants

viewed one of three arbitrary visual stimuli, each of which reliably predicted subsequent delivery of a pleasant glucose taste, an unpleasant salt taste, or a neutral taste. The time lag between 'stimulus presentation' and 'just before reward reinforcement', ranged from 4 to 10 seconds. Expectation of the sweet taste was associated with enhanced BOLD signal in the vicinity of the ventral striatum, dopaminergic midbrain, posterior dorsal amygdala, and OFC, while receipt of reward only engaged the OFC. These findings indicate that brain regions activated during reward expectation are somewhat dissociable from areas reacting to reward receipt, with the OFC mediating both processes (O'Doherty et al., 2002).

3.4.4 Imaging Feeding-Related Hormones

Animal research suggests that feeding is at least in part a bottom-up process whereby systemic signals target the central circuitry. Pharmacology paired with neuroimaging offers an innovative means of identifying the central targets of peripheral metabolic hormones, in humans. Presently, only leptin and peptide YY (PYY), homeostatic satiety signals derived from adipose tissue and gut, respectively, which target the brain, have been examined using this methodology. A detailed discussion of these three experiments follows.

Two studies have assessed the effects of leptin supplementation with functional imaging paired with food cues. In the first published experiment, Farooqi and colleagues demonstrated the importance of this peptide hormone in

feeding regulation using BOLD fMRI with food pictures. Two subjects with congenital leptin deficiency were evaluated pre- and post-treatment with recombinant human leptin, in both fasted and fed states. Leptin therapy significantly decreased energy intake at a test meal, reduced hunger scores in the fasting state and elevated satiety scores in the fed state. As compared with the satiated-deficient condition, ratings of the food images were also lower in the satiated-treated condition. The imaging data revealed prominent food cue-induced activation in the ventral striatum (anterior medial and posterior lateral) in the leptin-deficient state, which was reduced following leptin supplementation. Interestingly, the anterior medial response correlated positively with subjective ratings of liking of food pictures in both fasted and fed conditions pre-leptin, yet only in the fasted condition post-leptin (Farooqi et al., 2007). A second fMRI study also examined the effects of leptin therapy on the central response to food cues, this time in three leptin deficient adults. In this investigation, participants underwent three fMRI sessions. The first scan was after 57 months of continuous leptin therapy, thereafter, treatment was ceased for 33 days and a second fMRI scan was conducted. Finally, therapy was reinstated for 14 days followed by the final scan. All imaging data were acquired three-hours after an investigator-provided standard test breakfast. Results showed that leptin supplementation reduced food picture-induced hunger scores. Moreover, hormone therapy attenuated neural activity to food pictures in structures associated with hunger (insula, parietal and temporal cortex) while augmenting activity in structures associated with satiety and inhibition (prefrontal cortex) (Baicy et al., 2007).

Together, these two sets of imaging data urge that leptin modulates the sensitivity of striatal, insular, frontal, temporal and/or parietal cortices, to visual food stimuli. Further, the authors of the first experiment propose that exogenous leptin administration increases discrimination of the rewarding properties of food (cues) (Farooqi et al., 2007). While these studies provide compelling evidence that a homeostatic signal can modulate the 'extra (or non)-homeostatic' circuitry, they suffer from sample size limitations ($n \leq 3$). Conclusions must therefore be considered with caution.

A second anorectic hormone, PYY, has also been recently investigated in a randomized, double-blind, placebo-controlled study in food-deprived humans (Baicy et al., 2007). Neural responses in normal subjects (n=8) infused with PYY and saline (on separate days), in the absence of food-related sensory cues, were measured using functional MRI. In this experiment, it was assumed that increases in endogenous PYY concentrations following PYY infusion emulated the internal environment of the satiated state. Brain activation co-varied positively with plasma PYY concentrations in both homeostatic centers (hypothalamus, brainstem), and in areas implicated in reward, taste, memory and/or visual cue processing (substantia nigra/VTA, ventral striatum, caudolateral OFC, insula, parts of frontal/ partietal cortices). Further, data showed that caloric intake in a post-scan test meal correlated positively with hypothalamic signal in the control condition, and negatively with the left OFC in the PYY condition. The latter demonstrates that PYY-treatment shifted neural activation predicting food intake

from a homeostatic area (hypothalamus) to a reward area (OFC). This implicates the OFC in the feeding effects of PYY.

In sum, these imaging experiments indicate that circulating satiety factors PYY and leptin target both homeostatic and extra (or non)-homeostatic feeding centers in the brain to influence ingestive behavior, and that the extra (or non)-homeostatic regions likely mediate the effects of these hormones by modulating the hedonic/rewarding features of food. Indeed, peptide neuroimaging offers a novel approach to acquiring insight into both normal and diseased feeding regulation.

3.5 Summary

Human feeding research has profited from the discovery and implementation of functional neuroimaging techniques. Facilitating the real-time assessment of in vivo brain activity, this innovative tool has begun to expose the neuroanatomical substrates and underlying mechanisms that are involved in the regulation of eating behaviors. In this regard, several general conclusions can be extracted from the feeding-related PET and fMRI literature:

1. In addition to the traditional homeostatic regions (e.g. hypothalamus), an intricate and interconnected cortical-limbic-paralimbic network regulates appetitive behavior. Specific members of this circuit include areas associated with motivation, memory, reward, attention, emotion and

visual processing; namely, the amygdala, insula, striatum, OFC, midbrain and prefrontal and occipital cortex. These structures are responsive to food-related visual, gustatory and/or olfactory stimuli, and work in concert to orchestrate normal feeding responses (Figure 3-1).

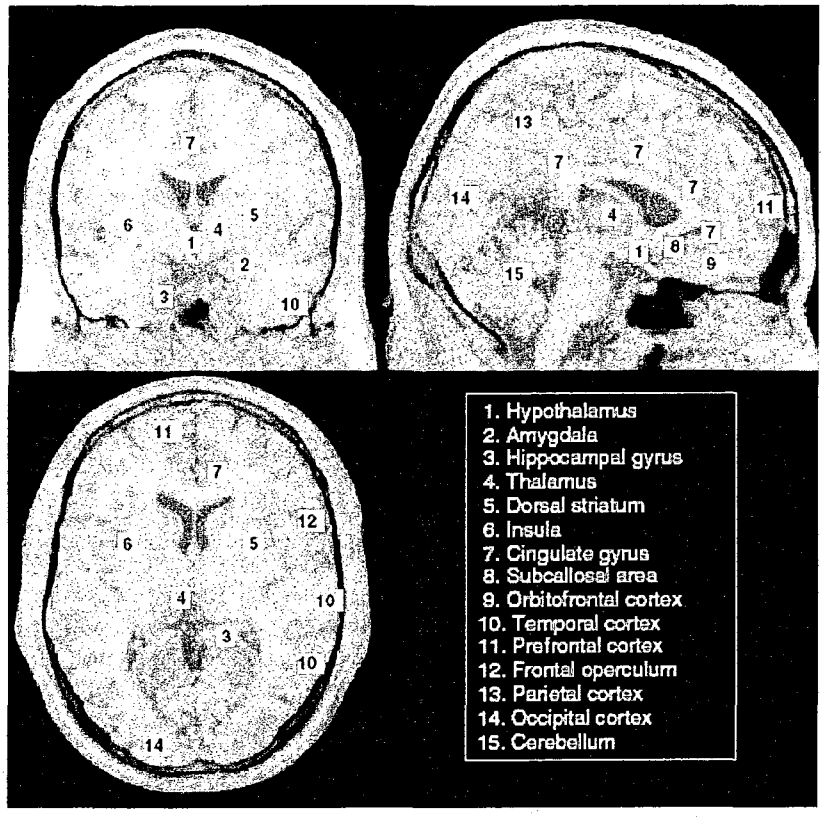
2. Food stimulation restrained to vision is an effective trigger of neuronal activation and is useful in the evaluation of human ingestive behavior. Further, food picture-induced activity of the CNS feeding network is modulated by both intrinsic factors (energy status) and extrinsic factors (hedonic value, dietary habits and affect). As well, brain responses to images of food in individuals with clinical conditions such as anorexia nervosa and obesity differ from those observed in healthy controls.

3. Preliminary evidence suggests that key components of the reward system are recruited during 'anticipation' of a pleasant taste reward.

4. Certain (typically) extra-homeostatic regions (OFC, striatum) are likely involved in the integration of selective metabolic hormones (leptin, PYY), and these interactions appear to influence the perception of food reward.

5. Dopaminergic neurotransmission (especially in the dorsal striatum) appears to participate in feeding-related responses.

Figure 3-1. Anatomical structures involved in the regulation of food intake are indicated in a coronal (upper left), saggital (upper right) and transverse (lower left) T1-weighted MRI images of the Montreal Neurological Institute's standard brain (Tataranni and DelParigi, 2003).



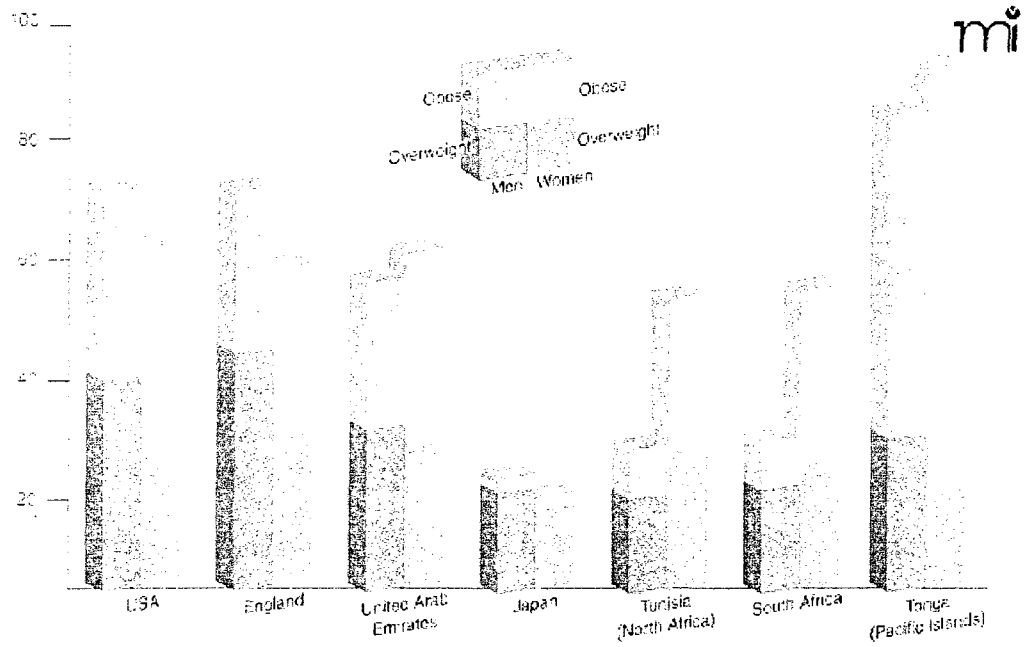
MODULE II:

Experiments

PREFACE MODULE II

The second module, consisting of two chapters, presents the experimental research conducted for this dissertation. As briefly mentioned in the *Introduction*, the rationale for these investigations emerges from the escalating incidence of obesity and its co-morbidities across the globe (Figure P2), and a lack of effective treatment programs (Mokdad et al., 2000; Mokdad et al., 2001; Mokdad et al., 2003; Ford et al., 2004; Bloom et al., 2008).

Figure P2. Prevalence of obesity in men and women among selected populations around the world. Rates of overweight (i.e. Body Mass Index between twenty-five and thirty) and obesity (i.e. BMI above thirty) can vary significantly within geographical regions according to several factors (e.g., ethnicity, see article for details). [Data from the International Obesity TaskForce. Data not standardized according to time period of collection or age ranges included] (Bloom et al., 2008).



Among the leading causes of illness and mortality, obesity is predominantly caused by eating calories in excess of the amount required for survival. One explanation for this apparently disadvantageous behavior is that genes that were beneficial under conditions of food shortage have become a liability in environments of plentiful cheap food (Ravussin and Bogardus, 2000; Prentice et al., 2005; Speakman, 2006). In other words, it would appear that our brains are *wired* to desire food. Therefore, there is much interest in comprehending how the brain regulates appetite and food consumption. In this regard, the current thesis studies sought to identify the neuroanatomical substrates responsive to visual food stimuli using fMRI, a modern tool for mapping activation patterns in the human brain, following *two* separate feeding-related protocols. Since both intrinsic and extrinsic factors have been demonstrated to influence ingestive behavior in humans, both were individually explored. In particular, the first project (Chapter 4) examined the brain response to visual food and non-food pictures following the administration of the metabolic hormone ghrelin, while the second project (Chapter 5) investigated the neural substrates of food reward expectation using a parallel stimulus paradigm. These two factors (i.e. ghrelin and expectation) have previously been demonstrated to participate in human feeding behavior (Wren et al., 2001a; O'Doherty et al., 2002), yet have never been researched using the 'fMRI-food picture' methodology. In fact, studies employing this methodology alone are few in number (Chapter 3, Section 3.4.1). The hypothesis and specific objective of each experiment is specified at the beginning of each chapter.

4

RESEARCH STUDY I

FOREWORD

Ghrelin is an orexigenic hormone that is mainly derived from the stomach. In both animals and humans, exogenous ghrelin treatment has been demonstrated to rapidly and potently stimulate food intake (Tschop et al., 2000; Wren et al., 2000; Wren et al., 2001a). Mechanistically, much evidence indicates that this metabolic signal regulates hypothalamic networks to promote nutrient consumption (Kamegai et al., 2000, 2001; Nakazato et al., 2001). Indeed, the hypothalamus, a classical CNS center for the homeostatic control of feeding and energy balance, is richly populated with receptors specific for the peptide. However, ghrelin receptors have also been identified in other brain areas including components of the reward system (Abizaid et al., 2006b; Zigman et al., 2006) and, local administration of the peptide into several neural structures associated with reward and motivation (e.g. nucleus accumbens and ventral tegmental area), induces

feeding (Carlini et al., 2004; Naleid et al., 2005; Abizaid et al., 2006b; Diano et al., 2006; Jerlhag et al., 2007). Notably, these findings have exclusively emerged from studies in rodents. In this regard, the aim of the following project was to test the hypothesis that (in addition to its effects on the hypothalamus), ghrelin modulates feeding behavior through the reward and motivation circuitry *in humans*. The specific objective was to measure the effect of exogenous ghrelin administration on the perception of food versus non-food images, using functional Magnetic Resonance Imaging, in order to expose the underlying activated neural networks. Indeed, this is the first human brain mapping study involving the administration of ghrelin.

This paper has recently been published in the journal *Cell Metabolism*. Supplemental data, research ethics board certificate, publication waivers and consent forms, are included in the Appendix. The approval letter from the Therapeutic Products Directorate of Health Canada along with the 'stability testing' data for the peptide are also appended. References for the manuscript are provided at the end of the chapter.

The title and contents of the article follow.

Ghrelin Modulates Brain Activity in Areas that Control Appetitive Behavior

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ABSTRACT

Feeding behavior is often separated into homeostatic and hedonic components. Hedonic feeding, which can be triggered by visual or olfactory food cues, involves brain regions that play a role in reward and motivation, while homeostatic feeding is thought to be under the control of circulating hormones acting primarily on the hypothalamus. Ghrelin is a peptide hormone secreted by the gut that causes hunger and food consumption. Here, we show that ghrelin administered intravenously to healthy volunteers during functional magnetic resonance imaging increased the neural response to food pictures in regions of the brain, including the amygdala, orbitofrontal cortex, anterior insula, and striatum, implicated in encoding the incentive value of food cues. The effects of ghrelin on the amygdala and OFC response were correlated with self-rated hunger ratings. This demonstrates that metabolic signals such as ghrelin may favor food consumption by enhancing the hedonic and incentive response to food-related cues.

INTRODUCTION

The presence of food, and the anticipation of pleasure it could provide, are potent triggers to feeding. This hedonic feeding behavior can be described as non-homeostatic in that it occurs in the absence of nutritional or caloric deficiency. While non-homeostatic feeding may have once provided an adaptive advantage to humans, in our plentiful environment it is likely a significant cause of obesity and its associated morbidity. Homeostatic feeding regulation mediated by the hypothalamus is well described (Saper et al., 2002); however, factors other than internal energy status also influence food intake. For instance, nutrient consumption is significantly influenced by external cues such as visual food stimuli. In animals, the behavioral response to such stimuli is mediated by specific neurons in the orbitofrontal cortex (OFC), amygdala, and striatum (Rolls, 1994; Holland and Gallagher, 2004), which form part of a meso-limbic reward system that is implicated in motivated behaviors (Cardinal et al., 2002). It has been suggested that while the hypothalamus primarily regulates the homeostatic drive to eat, these other neural circuits integrate environmental and emotional factors to control the 'hedonic' drive. Nonetheless, to influence behavior, homeostatic signals may access reward-related brain areas.

Ghrelin is a 28 amino acid peptide synthesized in the gastrointestinal tract that acts as a homeostatic signal involved in the brain-gut regulation of feeding (Kojima et al., 1999). Ghrelin administration increases food intake and adiposity in animals (Tschop et al., 2000; Nakazato et al., 2001). The preprandial rise and

postprandial fall in plasma ghrelin levels in humans suggests that it is a hunger signal that promotes meal initiation (Cummings et al., 2001). Administration of ghrelin to lean and obese subjects significantly increases energy consumed from a free choice buffet relative to placebo (Wren et al., 2001; Druce et al., 2005). Overall, acute and chronic nutritional states seem to influence endogenous levels of the peptide.

It is well established that ghrelin activates the hypothalamic NPY/AgRP orexigenic pathway (Nakazato et al., 2001), where ghrelin receptors are densely concentrated. However, ghrelin also has specific effects on many brain regions implicated in reward and motivation, including the ventral tegmental area (VTA), nucleus accumbens, amygdala and hippocampus (Carlini et al., 2004; Abizaid et al., 2006; Diano et al., 2006). The VTA and hippocampus express ghrelin receptors (Zigman et al., 2006), and direct injections into these regions as well as the amygdala lead to measurable changes at the neuronal and behavioral levels. Hence, it is possible that, in addition to its role as a metabolic signal for nutrient intake, ghrelin may modulate the incentive and hedonic aspects of ingestive behavior.

Here, we present evidence that ghrelin influences the responsiveness of brain regions involved in processing food cues in humans. We measured the cerebral response to food and non-food (scenery) images following single-blinded ghrelin infusions (1 μg/kg) (Figure 1), using functional magnetic resonance imaging

(fMRI). Twenty non-obese subjects were tested three hours after ingestion of a standardized meal. Twelve subjects viewed pictures before and after ghrelin administration (control/ghrelin group), and 8 subjects viewed the same pictures in two identical blocks without receiving ghrelin (control/control group). All subjects were told they might receive ghrelin. Ghrelin increased the response to food pictures in amygdala, OFC, insula, visual areas, and striatum. These regions encode the salience and the hedonic and incentive value of visual cues. This effect likely accounts for the ability of ghrelin to trigger and promote feeding.

EXPERIMENTAL PROCEDURES

Materials

Pharmaceutical-grade human ghrelin ($C_{149}H_{249}N_{47}O_{42}$, molecular weight (MW) = 3370.9) was purchased from CLINALFA, a subsidiary of Merck Biosciences AG (Laufelfingen, Switzerland). Manufactured according to GMP regulations, the peptide was sterile and pyrogen-free. The hormone was lyophilized in individual 100 μg glass vials and intended for intravenous infusion to human subjects.

Subjects

Twenty healthy medication-free normal weight male subjects were recruited. Twelve subjects participated in the control/ghrelin part of the study (mean age ± standard error of mean [SEM], 24.1 years ± 1.1; Body Mass Index ± SEM, 22.2 ± 0.5). Eight took part in the control/control study in which no ghrelin was administered (mean age, 23.2 years ± 1.3; Body Mass Index, 22.3 ± 0.7). All

were right-handed with normal or corrected-to-normal vision. Exclusion criteria included one or more of the following: history of neurologic or psychiatric illness, Body Mass Index > 25.9 or < 19, tobacco use, diabetes, gastrointestinal or eating disorders, food allergies, vegetarianism and/or contraindications for MRI scanning. The Dutch Eating Behavior Questionnaire (Van Strien et al., 1986), the Three Factor Eating Questionnaire (Stunkard and Messick, 1985), the Eating Attitudes Test (Garner et al., 1991) and the eating-related section of the Structured Clinical Interview for DSM-IV Screening Module (First et al., 1995), were used to exclude potential subjects with abnormal eating behavior. This research protocol was approved by Montreal Neurological Institute Research Ethics Board as well as by the Therapeutic Products Directorate of the Canadian government. Prior to the experiment, subjects were given a description of the paradigm and provided written informed consent.

Experimental Paradigm

All subjects underwent a single fMRI session at the Montreal Neurological Institute. On testing day, participants ate a standard test breakfast provided by us (125 ml orange juice, 42 g cheddar cheese, 2 slices toasted bread: 1 white and 1 whole wheat, 15 ml strawberry jam, 10 ml butter, 1 cup coffee with 20 ml 2% milk and 1 sachet white sugar) following a 12 hr overnight fast. Breakfast was taken at either 8 a.m. (n = 10) or 10 a.m. (n = 10), in our cafeteria, accompanied by one of the investigators. All subjects consumed the entire breakfast and

finished eating within 30 minutes. Visual analog scales rating hunger and mood were completed both before and after breakfast.

The imaging study was initiated three hours after the standardized breakfast to ensure that subjects were neither full nor hungry and lasted approximately 65 minutes. Ghrelin levels are at a nadir at this time (Cummings et al., 2001). Prior to subjects' placement in the scanner, an intravenous catheter was inserted into a left forearm vein and kept permeable with a slow infusion of normal saline. Following a high-resolution structural scan, the functional scanning began. The functional protocol was divided into two blocks (Figure 1). The first block entailed three five-minute functional runs (runs 1-3). During each run, 15 images (7-8 food, 7-8 scenery) were presented in random order. Subjects were instructed to focus their attention on the stimuli. Each picture was shown for 5 seconds followed by a 15 second dark screen with a central fixation cross. A total of 45 images were displayed (22 food, 23 scenery). At the start and end of the block, subjects answered questions regarding their mood and appetite (e.g. how hungry are you right now?) on a 10 point visual analog scale. Responses were recorded using a MRI-compatible mouse-like device. Images and questions were displayed on a projector screen using Presentation software (version 9.60, Neurobehavioral Systems, California, USA). Food and scenery pictures had been previously matched for visual appeal. The mean pleasantness ratings on a scale of 1-9 were, for food, 6.54 (sd: 1.55), and, for scenery, 6.57 (sd: 1.48).

Following the first image acquisition block there was a 20-minute period for ghrelin infusion during which no stimuli were presented. Subjects in the control/ghrelin group received two ghrelin infusions (0.5 μg/kg in normal saline infused over 60 s each time) approximately 13 minutes apart, in single-blinded fashion. Subjects in the control/control group did not. Prior to scanning subjects had been told they might or might not receive ghrelin during the scan, but not when this would occur if it did. The ghrelin was administered via the intravenous tubing from outside the scanner by an investigator who was not visible to the subjects. There was no change in the flow rate or temperature of the intravenous solution during ghrelin infusion.

The second block was identical to the first, consisting of three five-minute functional runs, except that different stimuli were used (23 food, 22 scenery). Questions regarding mood and appetite were again administered at the beginning and end of the block. All subjects viewed the same set of images. Pictures were presented in random order and no stimuli were repeated. Blood samples were collected just before the scanning started and as soon as it ended, to quantify glucose, insulin and growth hormone levels.

Finally, two post-scan tasks were administered to the subjects on a personal computer approximately 30 minutes later. First, subjects were shown all 45 food images they viewed in the scanner intermixed with 26 novel food images and asked to state whether or not they had seen each image while in the scanner. This

recognition task was performed to ensure that subjects were paying attention to the images during the scan. Second, they were asked to rate the images on a scale of 1 to 9 (1 = 'extremely dislike' and 9 = 'extremely like').

Imaging Parameters

Functional imaging data were acquired on a 1.5T Siemens Vision MRI scanner equipped with a quadrature radiofrequency head coil. Head motion was minimized with a vacuum cushion. First, high-resolution T1-weighted anatomical images were obtained. Thereafter, T2* weighted images with BOLD contrast were acquired. Thirty-two 4 mm thick slices that covered the whole brain were collected using the following parameters: T_R: 3 s, T_E: 40 ms, FOV: 256 mm, flip angle: 90° and voxel size: 4 x 4 x 4 mm. The functional session consisted of 6 runs of 5 min (3 control and 3 ghrelin, or 3 control 1 and 3 control 2), each consisting of 100 volumes per run. Food and scenery pictures were projected onto a screen in the scanner room and viewed through a mirror mounted on the head coil. Scanning time and stimulus presentation were synchronized by a trigger signal from the scanner at the beginning of every run. Two dummy images were taken at the onset of each sequence and discarded to reduce non-steady state effects.

Data Analysis

Functional images were spatially smoothed with a 6 mm Gaussian filter and motion corrected prior to statistical analyses. A general linear model was

designed using separate regressors for food and scenery pictures, consisting of boxcar functions convolved with a standard hemodynamic response function. Regional brain activation was determined by calculating a contrast of food minus scenery and computing effect and standard deviation at each brain voxel for each individual. These parametric images were transformed into Montreal Neurological Institute space (Collins et al., 1994) and a group analysis was performed using a mixed effects statistical model. The software package *fmristat* was used to conduct the statistical analysis (Worsley et al., 2002). The basic method is to calculate a t-statistic from the effect size and standard deviation of the general linear model for each individual. The t-value at each voxel is a measure of the likelihood that there was greater BOLD signal in response to the food than the scenery pictures at that location in the brain. Thus a t-map is generated. This map is then thresholded in order to correct for multiple comparisons based on the search volume (the entire brain), the amount of smoothing applied, and the degrees of freedom. Here we corrected for multiple comparisons by only listing brain regions containing clusters of voxels with $p < 0.001$ and a volume greater than 100 ml. This effectively reduces the risk of false positives to less than 1 in 20 (i.e. $p < 0.05$) for the experiment. Significant peaks are listed in tables along with the t-value and the location of the peak, expressed in Montreal Neurological Institute coordinates based on the stereotaxic atlas of Talairach and Tournoux (Talairach and Tournoux, 1988).

To confirm the significance of the ghrelin effect we performed an analysis of the interaction between group and condition. We did this by generating a t-map of the following effect: [(ghrelin - control) – (control 2 – control 1)]. Finally, we also created a t-map of the response to scenery pictures minus the response to the blank screen, to ensure that ghrelin did not have a non-specific effect on attention or arousal. We compared activation to the scenery pictures in the ghrelin and control states.

The effect sizes from the general linear model were also extracted from the peak voxels of areas of significant activation to food pictures, so that the ghrelin and control conditions could be compared, and correlated with behavioral data. Behavioral and hormonal data were analyzed using SPSS (SPSS Inc., Chicago, IL). A paired T-test was used to compare these measures in the ghrelin and control conditions.

Supplemental Data

Supplemental data include Supplemental Experimental Procedures, three figures and three tables and can be found in the Appendix.

RESULTS

Biochemical Data

All subjects had normal blood glucose prior to the scan. In the group that received ghrelin there was a significant increase in plasma growth hormone (pre-

scan ± SD: 1.0 ± 1.2 μg/l; post-scan: 62.7 ± 16.6 μg/l; $p < 0.001$), which is an expected consequence of the ghrelin infusions. In the control/control group there was also a significant increase in plasma growth hormone, but the effect was much smaller than in the ghrelin group (pre-scan: 0.14 ± 0.08 μg/l; post-scan: 4.99 ± 4.24 μg/l; $T = 3.02$, $p = 0.02$). Insulin levels did not change in either the control/ghrelin group (pre-scan: 37.0 ± 21.7 pmol/l; post-scan: 30.2 ± 16.2 pmol/l; $p = 0.24$) or the control/control group (pre-scan: 33.2 ± 12.30 pmol/l; post-scan: 23.5 ± 12.18 pmol/l; $T = 1.71$, $p = 0.13$).

Behavioral Data

In the control/ghrelin group there was a significant increase in the subjective ratings (mean ± standard error of the mean [SEM]) for hunger, and borderline increases for irritable and nauseous in the ghrelin relative to the control condition (hunger: control: 5.5 ± 0.6, ghrelin: 8.3 ± 0.4, $T = 4.91$, $p < 0.001$; irritable: control: 3.0 ± 0.5, ghrelin: 4.2 ± 0.7, $T = 2.74$, $p = 0.02$; nauseous: control: 1.3 ± 0.5, ghrelin: 2.4 ± 0.8, $T = 2.68$, $p = 0.02$; bored: control: 4.1 ± 0.6, ghrelin: 5.1 ± 0.6, $T = 1.45$, $p = 0.18$).

In the control/control group however the subjective rating for hunger did not change between the two blocks (hunger: control 1: 5.9 ± 0.68, control 2: 6.4 ± 0.83, $T = 1.08$, $p = 0.32$). There were increases of borderline significance in the subjective ratings for irritable and bored in the second relative to the first block of images (irritable: control 1: 3.5 ± 0.83, control 2: 5.0 ± 1.22, $T = 2.38$, $p = 0.05$;

bored: control 1: 4.9 ± 0.66, control 2: 6.5 ± 0.86, T = 3.36, p = 0.01). Nausea levels did not change (nauseous: control 1: 1.9 ± 0.63, control 2: 2.97 ± 0.94, T = 1.44, p = 0.19).

The food pictures were presented to the subjects a second time, after the scan outside the scanner. Food items presented in the ghrelin condition were more often recognized than those displayed in the control condition (mean ± SD: 88.8% ± 7.3 compared to 81.8% ± 10.8; T = 2.90, p = 0.01). There was no difference in the hedonic rating of the pictures viewed in the ghrelin versus the control condition (T = 0.73, p = 0.48). Note that there was no measurement of hedonic rating at the time of scanning however, so we cannot say whether ghrelin affected the perceived pleasantness of the food pictures during the scan.

In the control/control group there was no difference in the hedonic rating of the pictures viewed in the two control conditions (T = 0.81, p = 0.45) nor any difference in the recognition of food items presented in the two control blocks (control 1: 84.1% ± 3.3, control 2: 84.2% ± 5.3; T = 0.026, p = 0.98).

Neuroimaging Data (control/ghrelin group)

Neural activation associated with food stimuli was examined via subtraction of the scenery response (Table 1). In both the control and ghrelin states, visual areas in the parietal and occipital cortex were activated. However, the amygdala (bilaterally), right hippocampus and left pulvinar were more responsive to food

than scenery pictures only during the ghrelin condition. The anterior and mid-dorsal insula were also activated bilaterally in the ghrelin condition (Figure 2, Table 1). Extraction of the blood oxygen level dependent (BOLD) effect sizes from peak voxels identified in this contrast confirmed these findings (Figure 3). There was a statistically significant effect of ghrelin on the response in reward-related regions (bilateral amygdala, left OFC, right substantia nigra (SN)/VTA, left caudate, right hippocampus), anterior insular cortex (bilateral mid-dorsal and ventral insula), and visual areas (including pulvinar and fusiform gyrus).

A t-map of the food minus scenery contrast for all scans (ghrelin and control combined) was also generated. Significant activation was detected in bilateral caudolateral OFC, piriform cortex (olfactory area), and ventral pallidum, in addition to the aforementioned areas (Table 2).

To ensure that ghrelin did not alter the response to scenery pictures, scenery images were contrasted to the blank screen stimulus. Bilateral activation was observed in several occipital areas, namely the cuneus, fusiform, lingual and middle occipital gyri as well as in the pulvinar and parahippocampal gyrus. Activation was not different between the ghrelin and control conditions.

To determine whether the ghrelin effect observed here could play a role in promoting feeding we correlated the fMRI signal (the effect size from the general linear model) in the food minus scenery contrast with self-report measures. We

found that the increase in activation due to ghrelin correlated with self-reported hunger during the ghrelin scans in bilateral amygdala, left OFC and left pulvinar ($p < 0.05$, Figure 4). Ghrelin's effect on amygdala activation was also correlated with its effect on the left OFC ($p = 0.06$, Spearman's correlation) and the left pulvinar ($p = 0.03$, Figure 5). Finally, right insula activation correlated positively with recognition scores for the ghrelin pictures ($p = 0.05$).

Neuroimaging Data (control/control group)

In order to explore the possibility that the results in the group that received ghrelin were due to order effects, we subsequently recruited an additional 8 subjects who underwent the same paradigm with the exception that they only received normal saline rather than ghrelin. The two scanning blocks are referred to as control 1 and control 2. Neural activation associated with food stimuli was examined via subtraction of the scenery response. Regions belonging to significant clusters ($p < 0.05$, corrected for multiple comparisons) were identified. In control 2, several visual areas including the bilateral fusiform and occipital gyrus and left inferior parietal lobule were activated. Only the left inferior occipital gyrus was activated in the control 1. Importantly, no significant activation in the amygdala, insula, pulvinar, hippocampus, caudate or OFC was observed in either control conditions, even when lowering the threshold to $t = 2.5$ ($p = 0.005$ uncorrected). Extraction of BOLD effect sizes using peak voxel coordinates identified in the control/ghrelin group confirmed that there was no difference in the neural activation between control 1 and 2 in the aforementioned regions (all $p > 0.1$). A

t-map of the food minus scenery contrast for all scans (control 1 and control 2 combined) was also generated. Activation was detected in visual areas, including bilateral fusiform gyrus, and left insula (Supplemental Table S2). The peak voxel coordinates observed in the fusiform gyrus and insula were also used to extract the BOLD signal effect sizes in each of the two control conditions. Again, paired t-tests showed no difference between the two blocks (Supplemental Figure S3), confirming that the effects observed in the ghrelin group were not due to the order of conditions.

Finally, we generated a t-map of the interaction effect between the ghrelin – control group and the control – control group. There was a significantly greater increase in BOLD response (food minus scenery) in the control/ghrelin than the control/control group in the OFC, bilateral anterior insula, left mid-insula, left pulvinar, right SN/VTA, and bilateral fusiform (Supplemental Table S3). There were no areas showing a greater increase in the control 2 minus control 1 blocks than in the ghrelin minus control blocks.

Figure 1. Overview of the Protocol. (a) The fMRI session was 3 hr post-breakfast. (b) Three 5-minute functional runs with images were presented during each of the two blocks. In the ghrelin study, two ghrelin infusions (G1 and G2, 0.5 μg/kg each over 1 min, 15 minutes apart) were administered between the blocks. Subjects did not know whether or when ghrelin would be administered via the intravenous. The control study was identical except that no ghrelin was administered. Visual analog scales (VAS) assessing mood and appetite were administered at 4 time points. (c) Each run comprised 15 stimuli (half food, half scenes). Images were presented for 5 s followed by a 15 s fixation cross. Food and scenery images were presented randomly.

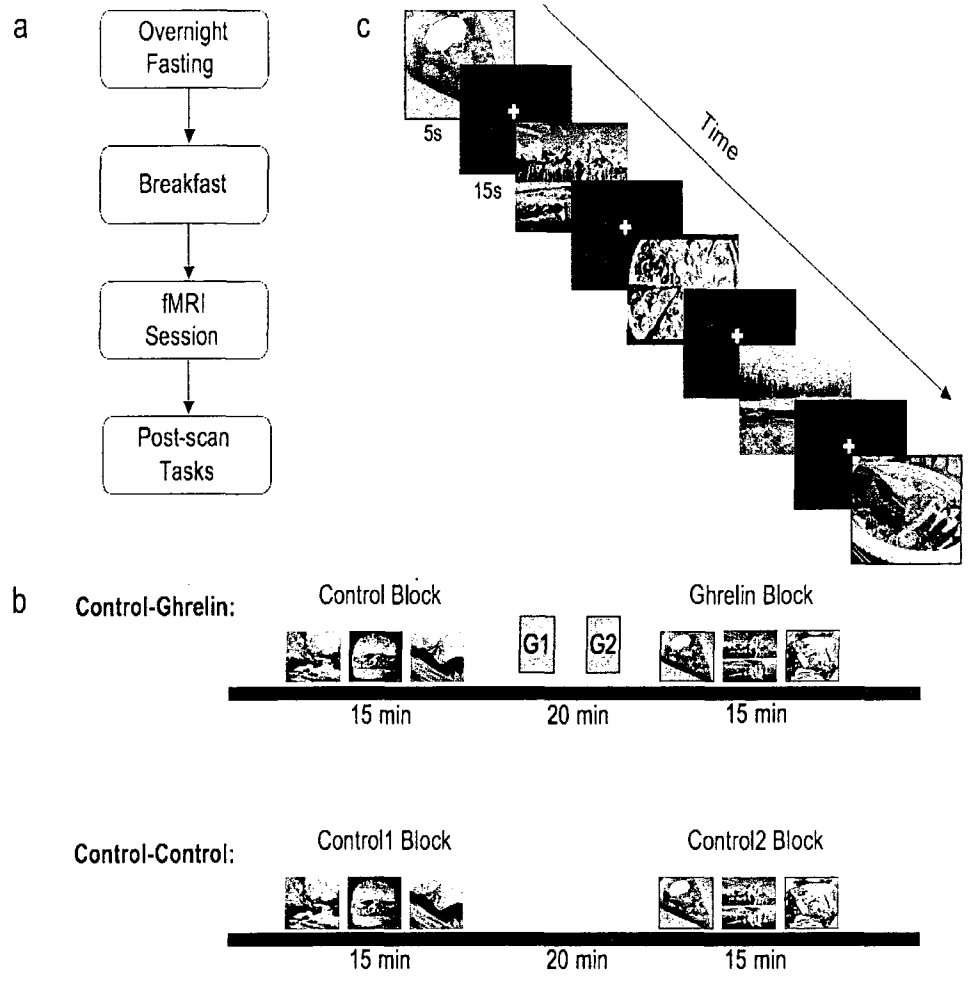


Figure 2. Statistical Maps. Representative T-maps for amygdala, fusiform gyrus, insula, pulvinar and OFC regions. All images are from the food minus scenery contrast, ghrelin condition (Table 1). The t-maps are thresholded at $t > 3$. Arrows indicate the peak locations for each region.

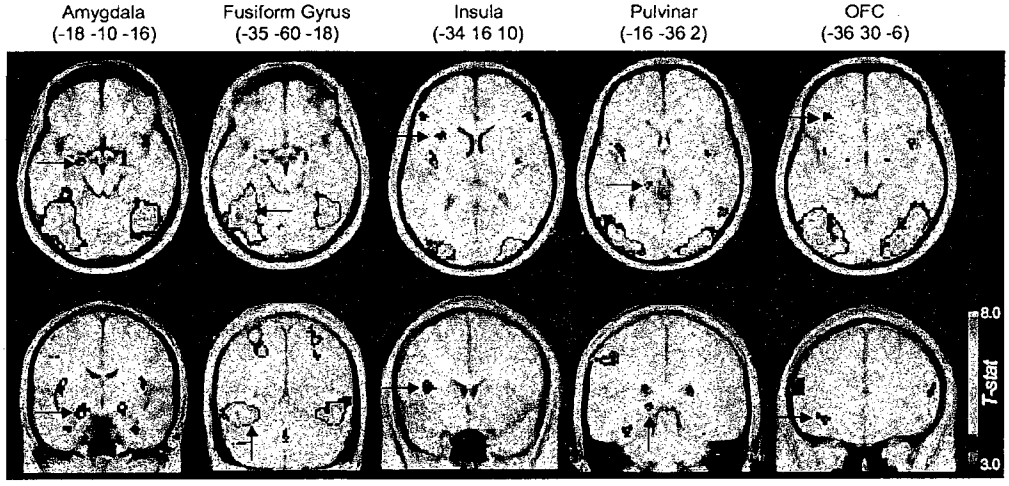


Figure 3. Ghrelin Effect. Bar graph showing the BOLD effect (parameter estimates from the general linear model of food pictures minus scenery pictures) in the ghrelin and control conditions for different regions identified in the categorical analysis. Error bars represent the SD of the general linear model. All comparisons show a significant effect of ghrelin ($p < 0.0001$, two-tailed), except for R occipital gyrus ($p = 0.0006$) and R mid-dorsal insula (not significant). Abbreviations and MNI coordinates: amygdala (right: 20, -10, -8; left: -18, -10, -16, OFC: orbitofrontal cortex (-36, 30, -6), SN/VTA: substantia nigra, ventral tegmental area (8, -16, -10), caudate (-8, -2, 12), hippocampus (32, -10, -30), Ins: insula (left anterior: -34, 16, 10; left mid-dorsal: -36, -12, 14; right mid-ventral: 42, 8, -6; right mid-dorsal: 42, -6, 10), occipital gyrus (right: 40, -67, -15; left: -51, -66, -10), left pulvinar (-16, -36, 2), left fusiform (-35, -60, -18).

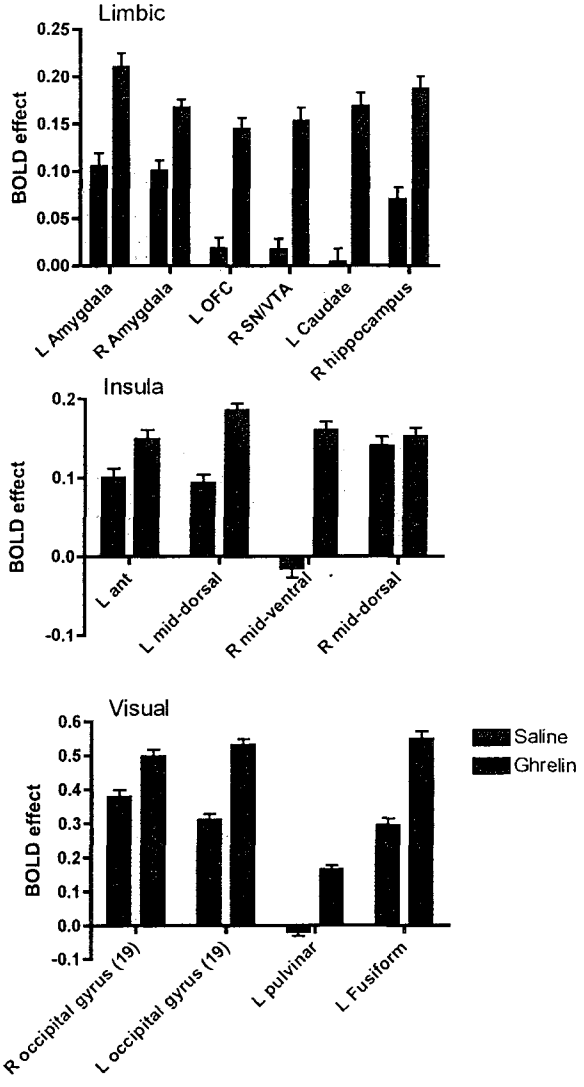


Figure 4. Hunger Effect. Correlation between mean self-rating of hunger during the ghrelin scans and the change in BOLD effect due to ghrelin (i.e., difference in parameter estimates of food minus scenery for the ghrelin and control scans at the peak voxel of this region). All regressions are $p < 0.05$ except for left amygdala ($p = 0.12$).

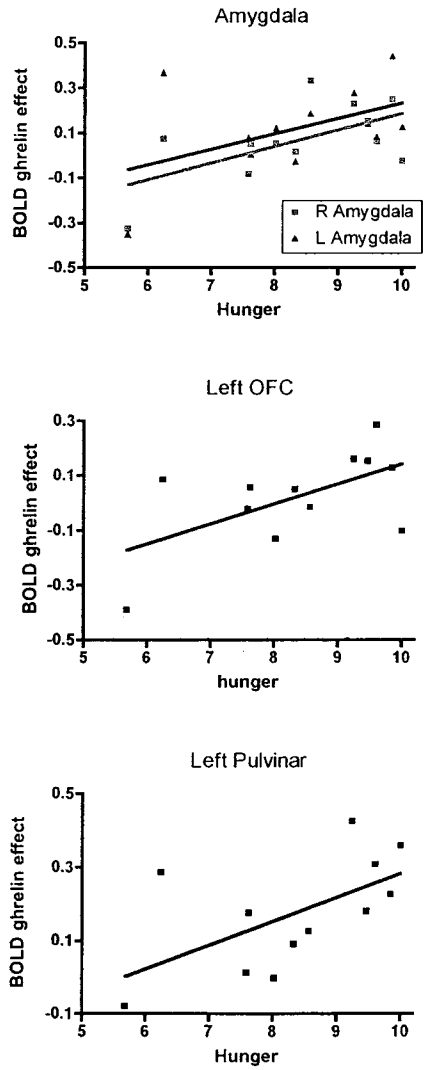
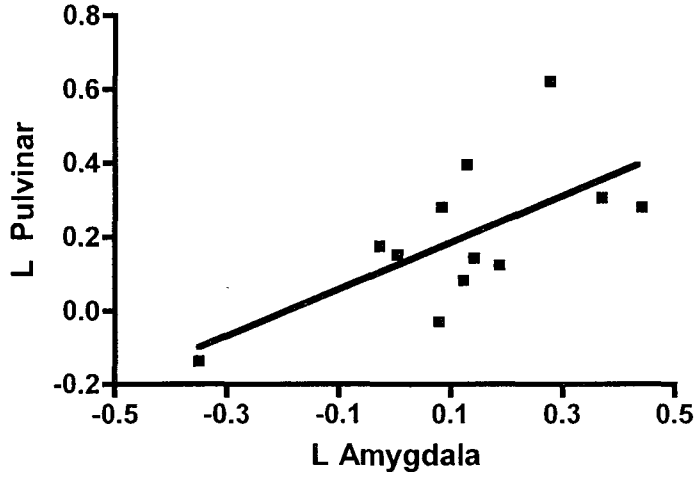


Figure 5. Correlations with Amygdala. Change in food minus scenery effect size (ghrelin minus control) for the left amygdala (x axis) and the left pulvinar and left OFC. Data were extracted from the peak coordinates in the subtraction analyses. Correlations were assessed using Spearman's rho. The p values for the correlations were 0.03 (pulvinar) and 0.06 (OFC).



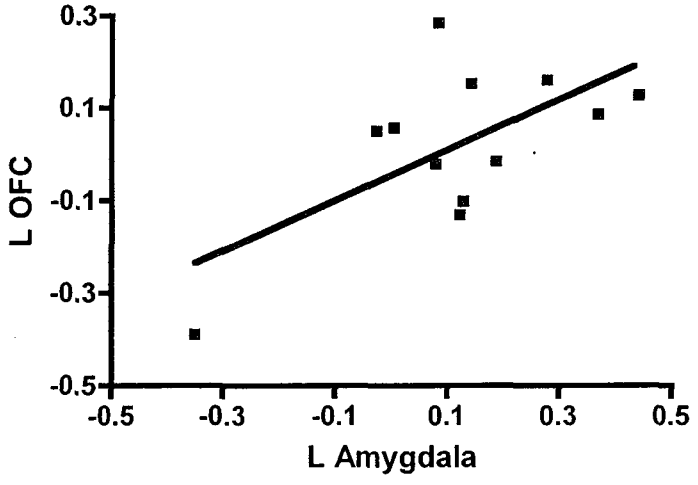


Table 1. Food minus Scenery Contrast for Ghrelin and Control Conditions. All peaks listed at $p < 0.001$ uncorrected with a minimum cluster extent of 100 mm^3. For the visual areas the extent of activation was quite large. When there is more than one peak within one functional region, only the most statistically significant peak is listed. The x, y, z refer to the coordinates in Montreal Neurological Institute space.

		GHRELIN				CONTROL			
Region		**t-stat**	**x**	**y**	**z**	**t-stat**	**x**	**y**	**z**
Orbitofrontal cortex	L	3.82	-36	30	-6				
Inferior/ middle frontal gyrus (6/44)	R	5.26	50	6	30	5.43	48	10	30
	L	4.75	-52	2	32	5.28	-44	4	32
Precentral gyrus	L	3.52	-50	4	4				
Amygdala	R	4.92	20	-10	-8				
	L	4.48	-18	-10	-16				
Hippocampus	R	4.13	32	-10	-30				
Insula (anterior)	R	4.24	42	8	-6				
	L	3.84	-34	16	10	4.2	-34	20	8
Insula (mid)	R	4.23	42	-6	10				
	L	5.92	-36	-12	14				
Caudate	L	3.59	-8	-2	12				
Cuneus	R	4.68	20	-100	4				
	L					4.77	-16	-100	-2
Fusiform gyrus	R	7.22	42	-70	-12	5.67	46	-72	-12
	L	8.64	-50	-66	-10	7.14	-34	-80	-14
Pulvinar	L	4.27	-16	-36	2				
Lingual gyrus	R	5.18	18	-98	-4				
	L	3.6	-10	-96	-12				

Inferior parietal lobule	L	5.36	-42	-48	58	6.21	-46	-38	50
Middle occipital gyrus	R	7.3	32	-90	6	4.91	38	-84	2
	L	6.05	-26	-92	14	6.85	-48	-74	-6
Superior occipital gyrus	L	5.53	-26	-76	30				
Superior parietal lobule	R	4.9	28	-58	56	5.4	32	-62	56
	L	6.36	-20	-66	48	5.56	-32	-58	56

Table 2. Food minus Scenery Contrast (Ghrelin and Control Conditions Combined). All $p < 0.05$ corrected except *$p < 0.001$ with a cluster size > 100 mm^3. BA: Brodmann area. OFC: orbitofrontal cortex. DLPFC: dorsolateral prefrontal cortex.

Region		t-stat	x	y	z
DLPFC	L	5.1	-52	34	18
DLPFC	R	3.89	54	30	18
OFC*	L	4.23	-28	30	-10
OFC*	R	3.81	25	28	-12
Insula (anterior)	L	5.31	-34	20	8
Inferior frontal gyrus	R	6.39	48	8	30
Medial frontal gyrus	L	4.13	0	8	54
Piriform cortex *	R	4.24	34	6	-14
Cingulate gyrus	L	4.71	0	4	40
Inferior frontal gyrus	L	6.38	-46	4	32
Insula (mid-dorsal)	R	5.13	42	-6	10
Precentral gyrus	L	4.42	-56	-6	42
Ventral pallidum	R	4.77	18	-10	-8
Ventral pallidum*	L	4.42	-24	-12	-10
Parahippocampal gyrus	R	4.55	36	-28	-22
Inferior parietal lobule	L	6.7	-46	-38	50
Inferior parietal lobule	R	4.15	32	-44	44
Superior parietal lobule	L	7.33	-32	-60	58
Superior parietal lobule	R	6.79	28	-60	56
Superior parietal lobule	L	6.29	-22	-66	50
Fusiform gyrus	R	8.85	38	-68	-14
Middle occipital gyrus	L	10	-50	-70	-10
Middle occipital gyrus	R	7.81	44	-74	-10
Middle occipital gyrus	L	5.03	-28	-74	28

Superior occipital gyrus	R	3.41	30	-80	26
Inferior occipital gyrus	R	8.25	40	-82	-8
Inferior occipital gyrus	L	11.22	-40	-86	-8
Inferior occipital gyrus	R	7.89	32	-90	0
Inferior occipital gyrus	L	7.13	-28	-92	12
Inferior occipital gyrus	L	7.95	-34	-94	0
Cuneus	R	5.13	24	-98	2
Lingual gyrus	L	5.08	-16	-98	-8
Lingual gyrus	R	5.03	16	-98	-2
Cuneus	L	4.86	-18	-100	-2

DISCUSSION

The cerebral response to food cues following ghrelin administration was increased in multiple areas, including the amygdala, insula, OFC and striatum, implicated in reward processing and appetitive behavior (Figure 3, Table 1). Moreover, self-reports of hunger were significantly increased in the ghrelin versus the control condition and correlated positively with the ghrelin-induced increase in cerebral activity in the amygdala, OFC and pulvinar (Figure 4). Finally, food pictures shown in the ghrelin condition were more easily recalled than those shown in the control condition. Importantly, these neural and behavioral changes were not observed in the double control experiment.

The brain regions reactive to ghrelin in this investigation play a role in the hedonic and incentive evaluation of visual stimuli. The amygdala is responsive to most biologically relevant stimuli and is crucially involved in the coordination of appetitive behaviors (Baxter and Murray, 2002; Cardinal et al., 2002; Holland and Gallagher, 2004). It is thought that the amygdala signals the current hedonic value of a stimulus or object, via interactions with the OFC (Holland and Gallagher, 2004), and that it increases the salience of biologically relevant stimuli by interacting with posterior visual areas (LaBar et al., 2001), such as the pulvinar and fusiform gyrus. Consistent with this model, we found that ghrelin's effect on left amygdala activation correlated with its effect on left OFC and left pulvinar activation (Figure 5).

Numerous studies in animals have shown that activity in amygdala and OFC signals the current appetitive value of a food or food cue (Baxter and Murray, 2002; Holland and Gallagher, 2004). Human imaging studies have confirmed this. When the hedonic/motivational value of an olfactory or visual cue is modulated using pleasant or unpleasant verbal labels (de Araujo et al., 2005), or by feeding an associated food to satiety (Gottfried et al., 2003), activity in amygdala and OFC, at coordinates close to the ones reported here, varies with pleasantness. The response of the OFC to food ingestion also decreases as a food is fed to satiety and its pleasantness decreases (Small et al., 2001; Kringelbach et al., 2003). The OFC and amygdala also mediate the anticipation and receipt of a taste reward (O'Doherty et al., 2002), and are additionally involved in the hunger-enhanced memory of food cues (Morris and Dolan, 2001). Correlated increases in the activity in the OFC and amygdala would therefore be expected in conjunction with an increase in hunger, as demonstrated here (Figure 4), and presumably food consumption.

The anterior insula was also ghrelin responsive. This structure, lying beneath the frontal operculum, includes the primary gustatory and visceral sensory cortex, and participates in several feeding-related functions (Scott and Plata-Salaman, 1999). In human imaging studies the insula responds to the taste of food (Small et al., 2001; O'Doherty et al., 2002) and to visual cues such as food pictures (LaBar et al., 2001; Simmons et al., 2005; St-Onge et al., 2005) and restaurant menus (Hinton et al., 2004), and this response varies with the subject's desire to eat

(Tataranni et al., 1999; Small et al., 2001; Hinton et al., 2004). Experiments with insula-lesioned rats show that the insular cortex functions in recalling changes in incentive value based on motivational state (Balleine and Dickinson, 2000). Therefore, like the amygdala and OFC, the anterior insula is involved in anticipation of food rewards and hedonic evaluation of food stimuli. The role of the anterior insula in incentive memory could account for the positive correlation between ghrelin-induced insular activation and subsequent recognition scores for food pictures in our study.

Ghrelin also increased the response to food pictures of brain areas involved in visual processing, attention, and memory. The pulvinar and fusiform gyrus are specifically involved in focused visual attention (Petersen et al., 1985; Kastner et al., 2004; Vuilleumier and Driver, 2007) and fMRI experiments show that the increased salience of behaviorally relevant or emotionally arousing visual stimuli is mediated by an interaction of amygdala, fusiform and pulvinar (Morris et al., 1997; Vuilleumier and Driver, 2007). We also found a ghrelin effect on the hippocampus, a structure that, along with the amygdala, is well known to be involved in memory formation (McGaugh, 2004; LaBar and Cabeza, 2006). Previous fMRI studies have shown activation of these two regions in response to food cues during the hunger state (LaBar et al., 2001; Morris and Dolan, 2001; St-Onge et al., 2005). Moreover, in animals, ghrelin regulates hippocampal spine synapse density and long term potentiation (Diano et al., 2006), and enhances spatial learning and memory (Carlini et al., 2002; Carlini et al., 2004).

Finally, two dopaminergic regions, the striatum and SN/VTA, were also modulated by ghrelin. These form the core of a reward network involved in the processing of feeding-related stimuli (Tataranni et al., 1999; Small et al., 2001; O'Doherty et al., 2002; Volkow et al., 2002; Small et al., 2003) and setting the motivational or incentive properties of food cues (Berridge and Robinson, 1998). Local injections of ghrelin into the rodent VTA promote locomotor activity, striatal dopamine release, and feeding (Abizaid et al., 2006; Jerlhag et al., 2007), while systemically administered ghrelin causes VTA dopamine neuron firing and simultaneous feeding behavior (Abizaid et al., 2006).

Ghrelin therefore appears to modulate the response to food cues of a neural network involved in the regulation of feeding and, most importantly, in the appetitive response to food cues. This appetitive response has several components: attention, anticipation of pleasure, motivation to eat (hunger), consumption, and memory for associated cues. Brain regions implicated in all of these functions were modulated by ghrelin. How ghrelin acts on the brain is not known, but several potential mechanisms have been identified. First, peripheral ghrelin may act on ghrelin receptors in the gut, which then relay information to the brain via the vagus nerve (Date et al., 2002), although this pathway is not necessary since total vagal deafferentation does not abolish the orexigenic effects of peripherally administered ghrelin (Arnold et al., 2006). This suggests that circulating ghrelin also acts directly on the brain. A likely region mediating this effect is the hypothalamus, where ghrelin increases the firing rate of NPY/AgRP

neurons in the arcuate nucleus (Nakazato et al., 2001). These neurons in turn project directly and indirectly to the VTA and amygdala (Saper et al., 2002; Kelley, 2004), where they act to regulate feeding behavior. Circulating ghrelin may also act directly on the dopamine system. There are ghrelin receptors in the VTA (Zigman et al., 2006) and peripheral ghrelin increases VTA dopamine neuron firing, an effect that is blocked by intra-VTA administration of a ghrelin receptor antagonist (Abizaid et al., 2006). Abizaid et al. also provide evidence that ghrelin increases the VTA response to appetitive stimuli. The effect of ghrelin on the amygdala could be direct, as the amygdala contains ghrelin-positive axon terminals (Cowley et al., 2003), or indirect via the hypothalamus or the VTA, which sends dopaminergic projections to the amygdala (Moore and Bloom, 1978). Note however that direct injection of ghrelin into the amygdala failed to increase food intake in one study (Carlini et al., 2004). Finally, the anatomical distribution of ghrelin receptors on presynaptic sites suggests that the hormone acts mostly as a neuromodulator, enhancing the response of neurons that control feeding (Cowley et al., 2003). Thus, while ghrelin itself may not directly initiate feeding, it likely enhances the appetitive response to food cues, as shown here.

We describe the effects of an orexigenic hormone, but two recent fMRI studies have examined hormones that reduce food intake. Leptin, when administered to two young individuals with congenital leptin deficiency, reduced the neural response to food pictures in the ventral striatum (Farooqi et al., 2007), an area associated with reward processing. We did not find a ventral striatal response to

food pictures in our study, although two functionally related regions, the SN/VTA and dorsal striatum, were sensitive to ghrelin. Note that our results are not inconsistent with those of Farooqi et al. since our subjects presumably had normal leptin levels, which appear to suppress the ventral striatal response to food pictures. Indeed, other fMRI studies have similarly failed to show ventral striatal activation in response to food pictures in healthy subjects (LaBar et al., 2001; Simmons et al., 2005). Another study measured the brain response to an infusion of PYY (Batterham et al., 2007), which is anorexic when administered systemically. Despite the differences in experimental paradigm, there was considerable overlap between the regions identified in that study and ours, possibly because PYY and ghrelin act on the same hypothalamic neurons (although with opposite effects). The left caudolateral OFC, SN/VTA, and left insula all showed a modulatory effect of PYY infusion.

Our results can also be compared to findings in Prader-Willi syndrome, a condition characterized by obesity, severe hyperphagia and persistent elevations in ghrelin levels. In a fMRI study, comparison of Prader-Willi patients to lean control subjects demonstrated an abnormally elevated response to food pictures, following a meal, in the amygdala, OFC, insula, parahippocampal gyrus, and fusiform (Holsen et al., 2006). Our results suggest that this represents an effect of ghrelin, which remains elevated after eating in these patients.

A few limitations must be addressed. First, it was not possible to counterbalance the control and ghrelin conditions, as ghrelin administered during the first block would have still had effects during a subsequent control block. We therefore performed a control experiment (control/control group) to confirm that the effects attributed to ghrelin were not merely due to scan order. This second group of subjects was recruited after the first study was completed, and their data were analyzed separately; however, the same scanner and analysis software were used. We also provide data from a separate experiment that did not have the potential confounding effect of order and confirms our findings (see Supplemental Data in Appendix).

Second, we failed to see any hypothalamic activation in our imaging data. The hypothalamus is densely populated with ghrelin receptors (Howard et al., 1996) and plays a crucial role in ghrelin-induced feeding behavior (Nakazato et al., 2001). It is possible that its small size may have impeded the detection of a change in BOLD signal. Note however that our study identified brain regions responding to food pictures. It is very likely that the hypothalamus affects the response of other brain areas to food pictures without itself displaying a change in neuronal firing when subjects view the pictures. There are also intrinsic limits to the fMRI method that must be taken into account. The spatial resolution of roughly 6mm does not permit us to identify the specific nuclei of the amygdala modulated by ghrelin. Moreover, signal dropout in the medial OFC means that we cannot exclude an effect in this region. Indeed, a study using positron

emission tomography, which does not suffer from signal loss in the OFC, demonstrated that a large part of medial OFC was involved in the appetitive response to chocolate ingestion (Small et al., 2001), along with the other regions identified in the current study. We may therefore have underestimated the spatial extent of the ghrelin effect in the OFC. Although we attribute the effects measured here to ghrelin, it is important to note that ghrelin causes increased secretion of growth hormone, ACTH, cortisol, and prolactin (Arvat et al., 2001), all of which may also act on the brain. Finally, since only males were included in this investigation, comparable studies in females must be pursued as there may be gender differences in food-related neural processing (Uher et al., 2006).

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5

RESEARCH STUDY II

FOREWORD

In humans, feeding is a very complicated behavior influenced by both internal homeostatic cues such as hormones, and external factors. While project one researched the cerebral response to food and control imagery following the administration of ghrelin, a circulating hormone known to participate in the homeostatic control of feeding, project two investigated the same response following manipulation of an extrinsic cognitive factor namely, expectation of food. Currently, just a single human imaging study has probed 'anticipation of reward' in a fashion that is directly relevant to feeding behavior (O'Doherty et al, 2002) (detailed discussion in Chapter 3, Section 3.4.3). In this study, O'Doherty and colleagues (2002) showed that while anticipation of a sweet taste reward (which was triggered by the presentation of a specific visual cue that signaled reinforcement with a glucose taste) elicited activation of several reward-related

areas namely, the amygdala, striatum, midbrain and OFC, receipt of the taste only engaged the OFC. These results imply that the brain regions mediating anticipation and receipt processes are partially separable. To the best of our knowledge however, no imaging investigation has explored the neural response to images of food while manipulating the state of expectancy. In this regard, the aim of the following experiment was to test the hypothesis that the state of food expectancy (i.e. 'expecting' food at the end of the scan versus 'not expecting' food at the end of the scan) modulates several of the brain regions involved in food reward. The specific objective was to identify the neuroanatomical substrates responsive to food and control pictures in the 'expecting' state and to compare them to those responsive in the 'not expecting' state, in energy-deprived male subjects, using functional Magnetic Resonance Imaging.

This article has been submitted to the journal NeuroImage for review. The ethics certificate, consent forms, and signed waivers from co-authors permitting inclusion of this manuscript into the present thesis, are provided in the Appendix. References for the article are provided at the end of the chapter.

The title and specifics of the paper follow.

State of Expectancy Modulates the Neural Response to Visual Food Stimuli in Humans

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ABSTRACT

Human brain imaging studies demonstrate distributed activation of limbic, paralimbic and sensory systems to food and food-associated cues. Activity in this circuit may be modulated by internal factors, such as hunger, and extrinsic factors. Anticipation to eat is one such factor, which likely impacts consummatory behavior. Here, the neural substrates of food expectancy were identified in ten healthy male participants who underwent two whole-brain functional Magnetic Resonance Imaging scans on separate days. Fasted subjects viewed images of food and scenery, in two counterbalanced states. During one condition, subjects were 'expecting' to eat right after the scan and during the other they were 'not expecting' to eat for 1 hour after the scan. Food pictures compared with scenery yielded bilateral activation in visual areas as well as in the left insula and amygdala in both conditions. The left dorsolateral prefrontal cortex, hippocampus and putamen were additionally activated in the 'not expecting' condition while right orbitofrontal cortex activity was enhanced in the 'expecting' condition. These data suggest that the areas engaged during the 'expecting' state are at least partially dissociable from those recruited in the 'not expecting' state, with areas of convergence in the amygdala and insula.

INTRODUCTION

Recent findings suggest that feeding regulation extends beyond the hypothalamus to include cortical, limbic and paralimbic structures (Tataranni and DelParigi, 2003). Among the regions previously implicated in the processing of food and associated stimuli such as food pictures are the amygdala, insula, striatum, midbrain, hippocampus and frontal cortex (Volkow et al., 2002; Killgore et al., 2003; Small et al., 2003; Wang et al., 2004; Porubska et al., 2006; Malik et al., 2008). These areas are extensively interconnected and mediate several aspects of reward processing.

Neuroimaging paired with food cues offers a novel approach to investigate human feeding behavior. Such studies indicate that food pictures activate components of the reward circuitry, a response that markedly differs from matched non-food control images (LaBar et al., 2001; Killgore et al., 2003; Simmons et al., 2005; Porubska et al., 2006; Uher et al., 2006; Cornier et al., 2007; Führer et al., 2008; Malik et al., 2008). Further, brain activation to food presentation in many reward-related structures, such as the amygdala, insula, and OFC, has been shown to be significantly enhanced when subjects are hungry, and reduced or absent following eating to satiety (LaBar et al., 2001; Morris and Dolan, 2001; Porubska et al., 2006). Homeostatic energy balance thus entails the integration of internal signals with external sensory inputs. However, appetitive status (i.e. hunger and satiety) is only one of several factors influencing the neural response to food pictures. The imaging data additionally reveal an effect of personality (Beaver et al., 2006),

affect (Killgore and Yurgelun-Todd, 2006, 2007), diet manipulation (Cornier et al., 2007), and the motivational salience of the food images themselves (Killgore et al., 2003). The contribution of cognitive factors, such as expectation to eat, on food-related neural activity remains elusive.

Anticipation of reward influences appetite regulation (Berridge, 1996) likely facilitating adaptive goal-directed behavior. In monkeys, expectancy of a reward subsequent to a behavioral response was preferentially associated with activity of dorsolateral prefrontal cortex (DLPFC) neurons (Watanabe, 1996). In humans, the expectation literature is limited. In fact, in the single relevant fMRI study, anticipation of a primary taste reward yielded significant activation of the OFC, the dopaminergic midbrain, the amygdala and striatum. Except for the OFC, a response was not observed in these structures during the receipt of the reward (O'Doherty et al., 2002).

The drug addiction literature suggests that expectation enhances responses to a reinforcer. For instance, ventral striatal dopamine release is enhanced in animals administered cocaine in a cocaine-paired environment relative to a novel environment (Duvauchelle et al., 2000). Similarly, human brain imaging studies have shown that expectation of various addictive drugs, including nicotine (Wilson et al., 2005; McBride et al., 2006) and cocaine (Volkow et al., 2003), modulated responses to drug-associated cues in several brain regions such as the DLPFC, OFC, anterior cingulate cortex, insula, thalamus and cerebellum.

Here, we examined the neural correlates of food expectation using visual food and non-food stimuli. Ten fasting male subjects underwent two fMRI scans, on separate days. During one scan they were 'expecting' to eat immediately after the scan and during the other they were 'not expecting' to eat for one hour after the scan. We hypothesized that several of the brain regions involved in food reward would be modulated by the state of expectancy.

METHODS

Subjects

Ten right-handed male subjects participated in this study (mean age ± SEM: 25.8 years ± 0.8). All participants were healthy with no history of psychiatric, neurologic, endocrine or gastrointestinal disorders, and no previous or current drug abuse. The Dutch Eating Behavior Questionnaire (DEBQ) (Van Strien et al., 1986) and the Three Factor Eating Questionnaire (TFEQ) (Stunkard and Messick, 1985) were administered to assess eating behavior. This research protocol was approved by the Research Ethics Board of the Montreal Neurological Institute. Prior to testing, subjects were given a description of the paradigm and provided written informed consent.

Experimental Procedures

Overview

All subjects underwent two functional Magnetic Resonance Imaging (fMRI) scans on separate days. On both days, subjects fasted for 8 hours prior to the scan

session. During one session they were expecting to eat immediately after the scan, and during the other they were told they could not eat for one hour after the scan. These two sessions were counterbalanced across participants. Scans took place from 16:00 - 17:30 or 17:30 - 19:00, with each subject tested at the same time of day.

Paradigm

Each imaging session comprised 8 functional runs and a T1-weighted anatomical scan. A run was 5 minutes in length and consisted of 15 images (7-8 food, 7-8 scenery). Images were displayed for 5 seconds followed by a 15 second fixation cross, using Presentation software (Version 0.76, Neurobehavioral Systems, California, USA). In total, 60 food and 60 scenery pictures were shown. The same images were presented in both sessions in a randomized fashion. Prior to the first functional run and at the end of each run subjects answered questions regarding their mood (bored, irritable) and appetite (hungry, craving food, craving dessert, want to eat, anticipating food). Responses were recorded using a MRI-compatible five button-press device. A response of 1 indicated "not at all" while 5 signified "extremely". At the end of both sessions, subjects were asked to rate the appeal of each food picture viewed during the scan on a scale of 1 to 9, with 1 being extremely dislike, and 9 being extremely like.

Imaging Parameters

Functional imaging data was acquired on a 1.5T Siemens Vision MRI scanner. Movement artifact was reduced via partial immobilization of the head with a head cushion. Following a fifteen minute high resolution T1 structural scan, T2* weighted images with blood oxygen level dependent (BOLD) contrast were collected. Thirty-two 4mm thick slices that covered the whole brain were obtained using the following parameters: T_R: 3 sec, T_E: 40 ms, FOV: 256 mm, flip angle: 90° and voxel size: 4 x 4 x 4 mm. The functional session consisted of 8 runs (8 x 5 minute (min) each), with one hundred volumes acquired in each 5 min sequence. Pictures were projected onto a screen in the scanner room and viewed through a mirror mounted on the head coil. Scanning time and stimulus presentation were synchronized by a trigger signal from the scanner at the beginning of every run. Two dummy images were taken at the start of each sequence and discarded to reduce non-steady state effects.

Data Analysis

Functional data were preprocessed prior to statistical analysis. The scans were smoothed with a 6mm full-width half-maximum Gaussian kernel and realigned to the third frame of each run. A general linear model was designed using separate regressors for food and scenery pictures, consisting of boxcar functions convolved with a standard hemodynamic response function. Regional brain activation was determined by calculating the contrast of 'food minus scenery' at each voxel and generating effect and standard deviation images for each individual, for each condition. A second GLM using separate regressors for low, high and medium

calorie foods was also designed. In this analysis, cerebral activation was determined by calculating contrasts of 'high minus low' and 'high minus medium and low'. All of the parametric images were transformed into Montreal Neurological Institute space (Collins et al., 1994) and a group analysis was performed using a mixed effects statistical model to generate t-maps. The software package *fmristat* was used to conduct the statistical analysis (Worsley et al., 2002). We report all peaks significant at $p < 0.05$, corrected for multiple comparisons, based on peak t-value and spatial extent. In addition, the effect sizes were extracted from the peak voxels demonstrating significant activation to food pictures so that the 'expecting' and 'not expecting' conditions could be compared.

Behavioral data were analyzed using SPSS 13.0. Paired t-tests were used to compare differences between 'expecting' and 'not expecting' conditions. $P < 0.05$ was considered significant. Regressions between regional brain activity and appetite-related behavioral scores were also performed in both states.

RESULTS

Behavioral Data

Paired t-tests (two-tailed) showed no difference between average scores ($\pm$ SEM) for hunger, craving food, craving dessert, anticipating food and want to eat across the 'expecting' (E) and 'not expecting' (NE) conditions (hunger: E: 4.53 ± 0.16, NE: 4.66 ± 0.19; $p = 0.458$; craving food: E: 4.52 ± 0.15, NE: 4.33 ± 0.24; $p =$

0.421; craving dessert: E: 3.32 ± 0.39, NE: 3.27 ± 0.37; p = 0.872; anticipating food: E: 4.69 ± 0.12, NE: 4.36 ± 0.31; p = 0.391; want to eat: E: 4.74 ± 0.076, NE: 4.64 ± 0.20; p = 0.581). However, subjects were slightly more bored and irritable in the 'not expecting' condition (bored: E: 2.21 ± 0.33, NE: 2.64 ± 0.33; p = 0.051; irritable: E: 2.20 ± 0.25, NE: 2.71 ± 0.32; p = 0.037).

Neuroimaging Data

Regions reported below belong to significant clusters ($p < 0.05$ corrected for multiple comparisons), unless otherwise specified.

Food minus Scenery Contrast

Areas Activated in Both 'Expecting' and 'Not Expecting' States

Neural activation associated with food stimuli was examined via subtraction of the scenery response (Table 1, Figure 1). In both the 'expecting' and 'not expecting' states, several visual areas in the parietal and occipital cortex were activated (clusters $p < 0.05$, corrected for multiple comparisons). Among these regions were the fusiform gyrus (bilaterally), cuneus (bilaterally), inferior occipital gyrus (bilaterally), middle occipital gyrus (right), and superior parietal lobule (bilaterally). The amygdala (left), insula (left), medial frontal gyrus (left), lateral premotor cortex (left) and inferior temporal gyrus (left) were also activated in both states. While peaks in the left mid-insula were significant, those in the left anterior insula did not reach statistical significance based on our criteria (Expecting, T = 3.91; Not expecting, T = 4.14).

Areas Activated Only in the 'Not Expecting' State

In addition to the areas specified above, activation in the DLPFC (left), hippocampus (left), extended amygdala (left), parahippocampal gyrus (left), anterior insula (right), ventral anterior insula/ piriform (left), piriform cortex (left), putamen (left), superior temporal gyrus (left), superior frontal gyrus (left) and ventrolateral prefrontal cortex/opercular area (right), was exclusively observed in the 'not expecting' condition (Figure 2).

Areas Activated Only in the 'Expecting' State

The right inferior temporal gyrus and the right mid-insula were additionally activated in the 'expecting state'. However, the insula activation was sub-threshold (T = 4.65).

Areas Activated in 'Expecting' and 'Not Expecting' States Combined

A t-map of the food minus scenery contrast for 'all' scans ('expecting' and 'not expecting' conditions combined) was also generated (Table 2). Activation was detected bilaterally in the amygdala, insula, ventral global pallidus and orbitofrontal cortex. Unilateral responses were observed in the hippocampus (left), lateral premotor area (Brodmann area 6, left), pulvinar (left) and thalamus (ventral lateral nucleus, right). As before, extensive occipital–parietal activation was present.

Expectancy Effect

Extraction of the BOLD effect sizes from peak voxels identified in the food minus scenery contrast demonstrated significantly increased neural activation in 'not expecting' relative to the 'expecting' condition, in the left dorsolateral prefrontal cortex (x, y, z: -38, 30, 18; T(9) = -3.113, p = 0.012) and in the right anterior insula (x, y, z: 30, 16, 4; T(9) = -2.336, p = 0.044) (Figure 3). The coordinates used were from the 'not expecting' t-map as there was no similar area activated in the 'expecting' state. Conversely, the right orbitofrontal cortex (x, y, z: 24, 26, -12) showed significantly greater activation in the 'expecting' condition (T(9) = 2.997, p = 0.015). Coordinates used were derived from the 'all' conditions t-map. These are the only voxels activated by the food pictures that showed a statistically significant effect of condition.

Regression

Right anterior insula activation was significantly predicted by average *hunger* score in the 'not expecting' condition ($R^2 = 0.501$, F = 8.026, p = 0.022). Average *want to e*at score also predicted activation in this region ($R^2 = 0.637$, F = 14.010, p = 0.006).

Scenery minus Baseline Contrast

To ensure that the state of expectancy did not alter the response to scenery images, a scenery minus baseline (blank screen) contrast was performed. Scenery pictures activated the cuneus, fusiform gyrus, inferior occipital gyrus, lingual gyrus, middle occipital gyrus, parahippocampal gyrus, posterior cingulate,

superior parietal lobule and thalamus. The majority of the regions exhibited a bilateral response, with no significant differences between the two conditions (Table 3).

Scenery minus Food Contrast

Subtracting out the food-related activation from the cerebral response to the scenery images, significant and mostly bilateral neural activity was observed in the lingual gyrus, parahippocampal gyrus, posterior cingulate, precuneus and fusiform gyrus, in both conditions. The middle frontal gyrus (R), occipital gyrus (bilateral) and cuneus (bilateral) were additionally activated in the 'not expecting' condition (Table 4).

Calorie Analysis

Although not an original aim of the study, the specific neural response to low, medium and high calorie food images in the 'expecting' and 'not expecting' conditions was examined. Data was also collapsed across the two conditions and the calorie-related neural activation again observed.

Contrasts of interest were the following:

1. High calorie minus low calorie: 'expecting' versus 'not expecting' (Table 5).
2. High calorie minus low calorie: all data (Table 6).

3. High calorie minus medium and low calorie: 'expecting' versus 'not expecting' (Table 7).
4. High calorie minus medium and low: all data (Table 8).
5. Low calorie minus high calorie: 'expecting' versus 'not expecting' (Table 9).
6. Low calorie minus high calorie (all data): no significant clusters.

As this experiment was not designed to look at how the state of expectancy influences the neural response to images of variable caloric content, an equal number of low and high calorie images were not employed. Hence, there is insufficient power to draw any firm conclusions from the attained results. For this reason, this data is not discussed any further.

FIGURE 1

Behavioral scores across 'expecting' and 'not expecting' functional MRI scans. Bar graph represents mean scores ± SEM. Responses were integers from 1 to 5, with 5 being the maximum. The sample size was 10 for all measures except anticipating food (n=9). Paired t-tests (two-tailed) showed a significant difference between the two conditions for the bored and irritable measures only. *P < 0.05.

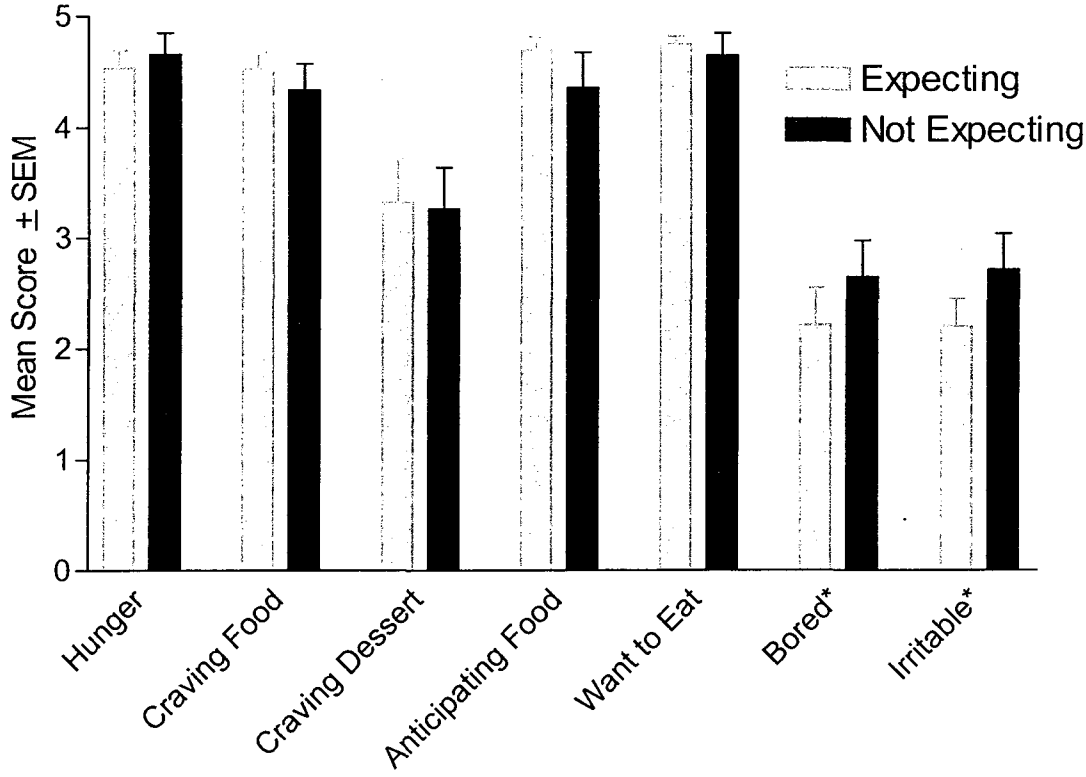

FIGURE 2

Representative T-maps for amygdala, insula and fusiform gyrus regions. Images are from the food minus scenery contrast, 'expecting' (E) and 'not expecting' (NE) conditions (Table 1). Coronal and verticofrontal slices for each area are shown. MNI coordinates: left amygdala (E: -20, -6, -12; NE: -22, -2, -18), left mid-insula (E: -40, -4, -2, NE: -38, -6, 6), left fusiform gyrus (E: -34, -54, -18, NE: -34, -50, -18). The t-maps are thresholded at $t > 3$.

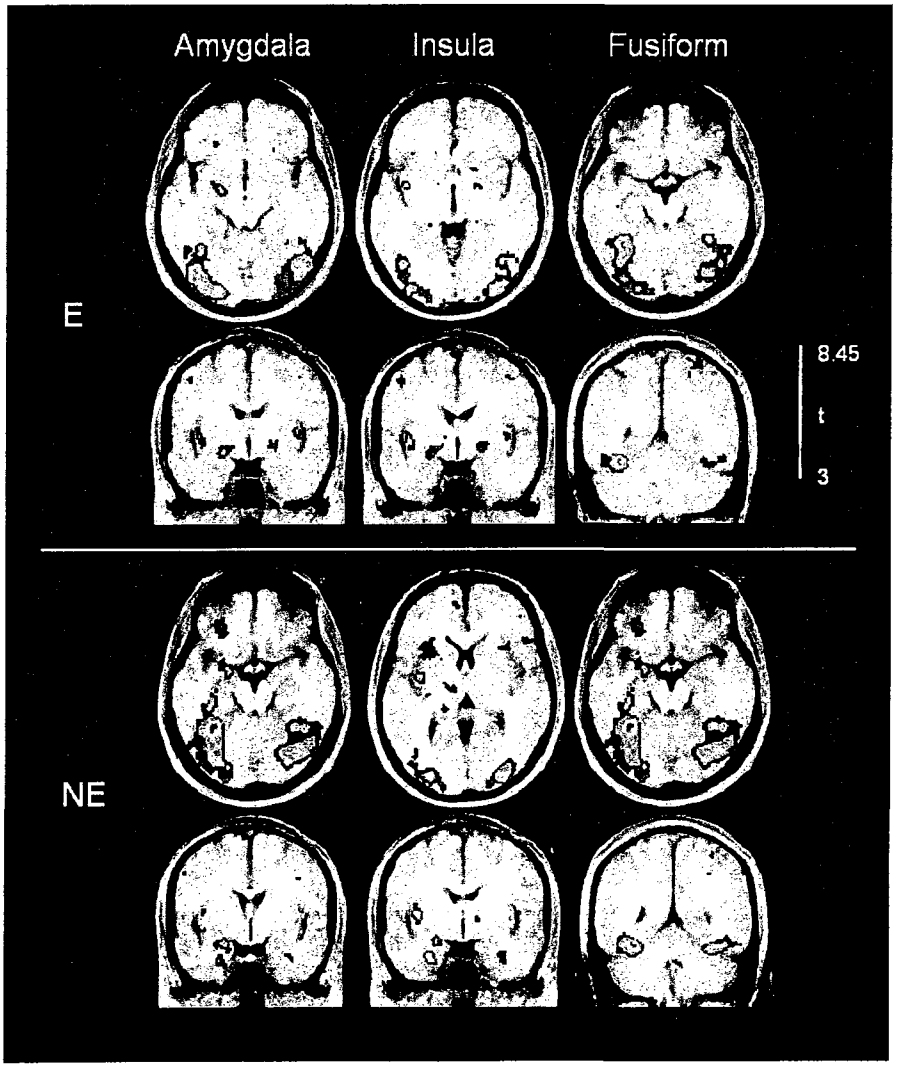

FIGURE 3

Statistical T-maps for dorsolateral prefrontal cortex, insula, and hippocampus. All images are from the 'not expecting' condition, food minus scenery contrast (Table 1). MNI coordinates: left dorsolateral prefrontal cortex (DLPFC: -38, 30, 18), right anterior (ant) insula (30, 16, 4), left hippocampus (-32, -12, -26). The t-maps are thresholded at $t > 3$.

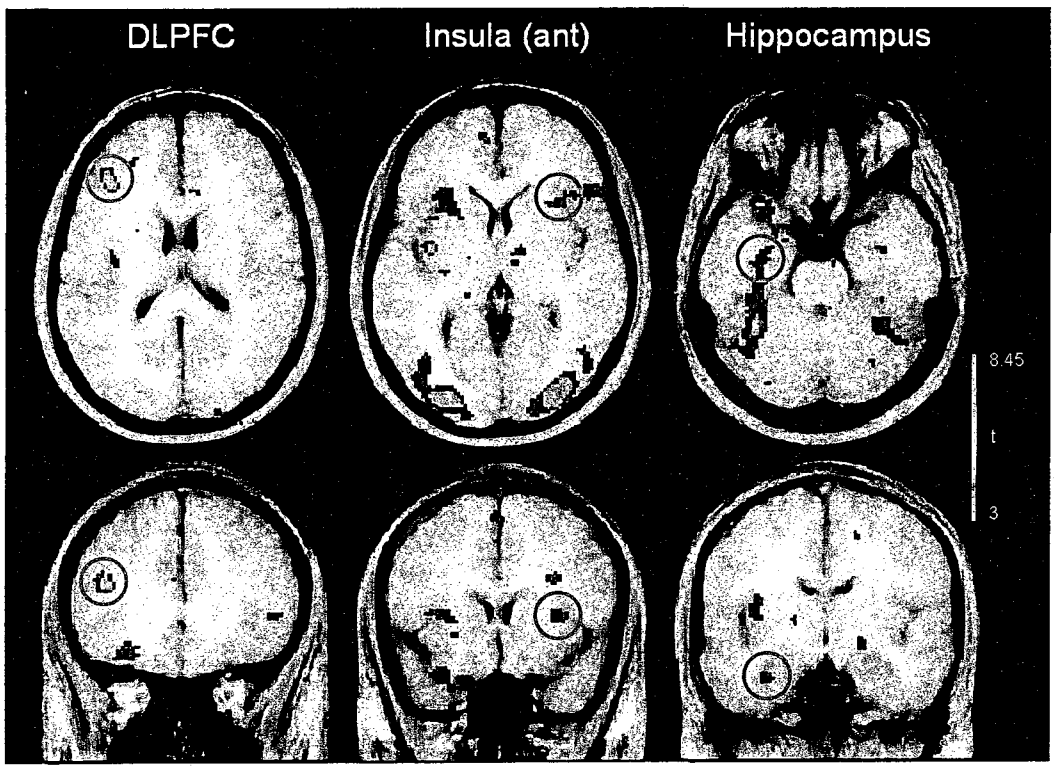

FIGURE 4

Expectation Effect. Bar graph showing the BOLD effect (parameter estimates from the general linear model of food pictures minus scenery pictures) in the 'expecting' and 'not expecting' conditions, for two regions identified in the categorical analysis. Abbreviations and MNI coordinates: DLPFC: dorsolateral prefrontal cortex (left: -38, 30, 18), anterior (ant) insula (right: 30, 16, 4), OFC: orbitofrontal cortex (right: 24, 26, -12). *P < 0.05.

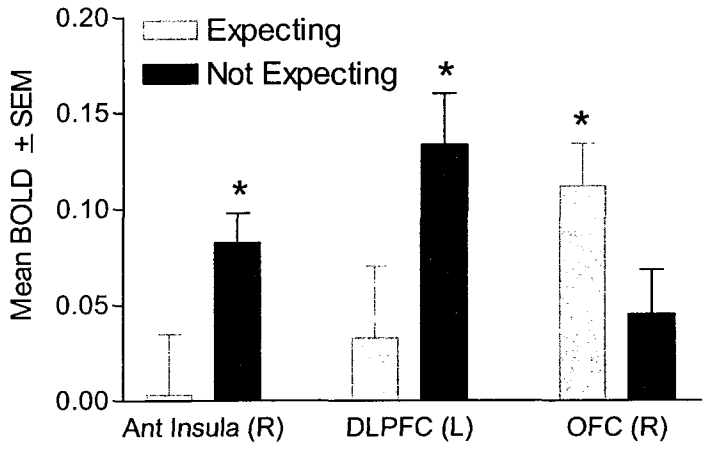

TABLE 1. Food minus scenery contrast for 'expecting' and 'not expecting' conditions. Listed peaks below belong to significant clusters ($p < 0.05$, corrected for multiple comparisons). DLPFC: dorsolateral prefrontal cortex. *Cluster not significant, but apriori region.

Region		Expecting				Not Expecting			
		T-stat	x	y	z	T-stat	x	y	z
Amygdala	L	4.66	-20	-6	-12	5.37	-22	-2	-18
Cuneus	R	4.71	24	-98	12	5.82	28	-92	0
	L	4.79	-20	-100	12	4.79	-20	-94	-2
Fusiform Gyrus	R	5.13	36	-50	-18	6.92	52	-62	-14
	L	8.45	-34	-54	-18	8.0	-34	-50	-18
Inferior Occipital Gyrus	R	5.52	48	-78	-10	7.43	40	-80	-6
	L	6.52	-34	-86	-12	6.58	-36	-86	-10
Inferior Temporal Gyrus	R	4.47	52	-58	-16				
	L	4.78	-46	-62	-6	5.29	-46	-60	-10
Insula (middle)	R	*4.65	40	-6	4	-	-	-	-
	L	4.95	-40	-4	-2	5.25	-38	-6	6
Insula (anterior)	R	-	-	-	-	4.2	30	16	4
	L	*3.91	-34	14	4	*4.14	-26	16	4
Medial Frontal Gyrus	L	3.97	-4	-2	70	4.48	-2	4	62
Lateral Premotor Cortex	L	4.36	-40	6	32	4.28	-52	8	24
Middle/Inferior Occipital Gyrus	L	5.41	-40	-72	-6				
Midline Medial Frontal Gyrus		3.81	0	0	66				
Superior Parietal Lobule	R	4.64	24	-60	62	4.27	32	-54	60
	L	4.33	-22	-66	48	4.4	-20	-64	52
DLPFC (BA 46)	L					5.71	-38	30	18
Extended Amygdala	L					4.13	-26	4	-22
Ventrolateral PFC (opercular area)	R					3.99	42	22	2

Hippocampus	L					4.14	-32	-12	-26
Insula (ventral ant)/ Piriform	L					4	-28	14	-24
Parahippocampal Gyrus	L					5.2	-28	-6	-30
	L					4.48	-24	-6	-32
Piriform Cortex (1° olfactory area)	L					4.05	-28	2	-20
Putamen	L					3.5	-22	12	4
Superior Frontal Gyrus	L					3.73	-4	2	70
Superior Temporal Gyrus	L					4.87	-34	18	-26
Middle Occipital Gyrus	L					5.01	-28	-90	4
	R	7.08	52	-72	-6	7.79	48	-64	-4

TABLE 2. Food minus scenery contrast for 'expecting' and 'not expecting' conditions combined (all data). Listed peaks belong to significant clusters ($p < 0.05$, corrected for multiple comparisons).

Region		T-stat	x	y	z
Amygdala	R	4.88	20	-10	-8
	L	5.44	-22	-6	-16
Cingulate Gyrus	R	3.59	4	12	40
	L	5.44	-4	2	28
Cuneus	L	5.23	-16	-100	0
	R	5.87	22	-98	12
Fusiform Gyrus	R	8.14	50	-64	-14
	L	11.3	-34	-54	-18
Hippocampus	L	4.85	-34	-24	-14
Inferior Frontal Gyrus	R	3.91	42	6	34
	L	3.84	-46	38	8
Inferior Occipital Gyrus	R	9.02	42	-84	-4
	L	10.18	-34	-86	-10
Inferior Parietal Lobule	L	5.73	-50	-40	48
Insula	R	6.72	40	-8	6
	L	6.94	-38	-4	-2
Lateral Premotor Area (BA6)	L	4.64	-52	4	40
Lingual Gyrus	R	4.1	14	-88	-14
Medial Frontal Gyrus	R	4.83	2	4	62
Middle Occipital Gyrus	R	9.2	50	-68	-6
	L	6.64	-50	-60	-8
Middle/Inferior Frontal Gyrus	L	4.06	-40	38	20
Midline Medial Frontal Gyrus	L	4.99	0	0	64

OFC	R	4.6	24	26	-12
	L	4.05	-28	34	-14
OFC/Inferior Frontal Gyrus	L	3.88	-22	28	-6
Parahippocampal Gyrus	R	3.84	16	-4	-10
	L	4.37	-32	-36	-24
Precuneus	R	4.51	28	-46	40
Pulvinar	L	5.81	-16	-34	2
Superior Parietal Lobule	R	6	26	-62	64
	L	6.19	-20	-62	52
Superior/Medial Frontal Junction	R	3.82	8	20	58
	L	5.33	-4	0	70
Thalamus (Ventral Lateral Nucleus)	R	4.5	14	-8	2
Uncus	R	5.04	32	-8	-30
Ventral Globus Pallidus	R	4.99	18	-12	-8
	L	5.03	-12	-8	-6

TABLE 3. Scenery minus baseline contrast for 'expecting' and 'not expecting' conditions. Listed peaks belong to significant clusters ($p < 0.05$, corrected for multiple comparisons). *Cluster not significant ($p > 0.05$) but peak for this region present.

Region		Expecting				Not Expecting			
		T-stat	x	y	z	T-stat	x	y	z
Cuneus	R	5.14	20	-98	10	6.15	22	-96	2
	L	7.96	-10	-100	0	9.28	-10	-100	0
Fusiform Gyrus	R	8.61	28	-52	-8	8.73	28	-52	-8
	L	8.6	-28	-70	-16	9.61	-28	-56	-10
Inferior Occipital Gyrus	R	6.38	28	-84	-10				
	L					6.29	-36	-84	-14
Lingual Gyrus	R	7.33	16	-88	-6	7.71	16	-88	-6
	L	8.02	-12	-98	-4	8.97	-10	-96	-6
Middle Occipital Gyrus	R	8.68	36	-84	10	7.77	38	-86	18
	L	7	-24	-96	8	7.3	-34	-88	16
Parahippocampal Gyrus	R	10.52	22	-40	-14	9.68	22	-40	-14
	L	8.15	-24	-50	-6	9.89	-28	-52	-6
Posterior Cingulate	R					4.53	16	-50	12
	L	4.14*	-20	-62	12				
Superior Parietal Lobule	L	3.64*	-26	-70	36	4.34	-22	-68	40
Thalamus	R	5.74	24	-32	2	5.97	22	-30	0
	L	4.9	-22	-32	0	5.95	-22	-34	2

TABLE 4. Scenery minus food contrast for 'expecting' and 'not expecting' conditions. Listed peaks belong to significant clusters ($p < 0.05$, corrected for multiple comparisons).

Region		Expecting				Not Expecting			
		T-stat	x	y	z	T-stat	x	y	z
Cuneus	R					3.58	12	-82	20
	L					4.55	-2	-78	6
Fusiform Gyrus	L	6.54	-26	-50	-4	6.97	-28	-52	-2
Lingual Gyrus	R	4.76	18	-58	0	4.64	10	-74	4
	L	4.61	-18	-54	0	5.78	-12	-58	6
Middle Frontal Gyrus	R					5.11	24	24	44
Occipital Gyrus	R					5.43	10	-48	6
	L					5.6	-10	-48	2
Parahippocampal Gyrus	R	6.94	26	-52	-4	5.11	18	-44	-6
	L	5.15	-18	-44	-10	3.98	-18	-44	-10
Posterior Cingulate	R	5.7	14	-58	10	6.9	14	-58	14
	L	5.11	-20	-62	14	5.61	-14	-60	8
Precuneus	R	5.34	24	-64	20	5.67	12	-64	20
	L					5.24	-20	-64	20

TABLE 5. High minus low calorie contrast for 'expecting' and 'not expecting' conditions. Listed peaks belong to significant clusters ($p < 0.05$, corrected for multiple comparisons, $*p_{cluster} = 0.058$).

Region		Expecting				Not Expecting			
		T-stat	x	y	z	T-stat	x	y	z
Fusiform Gyrus	R	5.26	52	-64	-20	*4.7	44	-66	-16
	L					4.52	-26	-54	-12
Middle Occipital Gyrus	R	4.02	36	-76	28	4.02	50	-60	-4
	L					4.92	-46	-68	-6
Middle Temporal Gyrus	R	3.39	36	-70	26				
Superior Parietal Lobule	R					4.57	26	-62	64
Middle Temporal Gyrus	R					3.89	54	-66	6
Inferior Temporal Gyrus	R					3.64	54	-68	0
Cerebellum	L					3.78	-16	-62	-20

TABLE 6. High minus low calorie contrast for 'expecting' and 'not expecting' conditions combined (all data). Listed peaks belong to significant clusters ($p < 0.05$, corrected for multiple comparisons).

Region		T-stat	x	y	z
Fusiform Gyrus	R	5.05	48	-64	-16
	L	4.9	-36	-64	-14
Inferior Occipital Gyrus	R	4.81	44	-76	-4
	L	3.88	-42	-80	-16
Middle Occipital Gyrus	L	6.19	-48	-72	-4
	R	4.43	48	-64	-6
Inferior Temporal Gyrus	R	3.79	52	-68	-2
Middle Temporal Gyrus	R	4.17	34	-80	24
Precuneus	R	4.63	24	-78	46
Superior Parietal Lobule	R	5.11	28	-62	62
Superior/Inferior Parietal Lobule	R	3.38	38	-52	58
Cerebellum	L	3.86	-16	-62	-20

TABLE 7. High minus medium and low calorie contrast for 'expecting' and 'not expecting' conditions. All peaks listed belong to significant clusters ($p < 0.05$, corrected for multiple comparisons). There were no significant clusters in the expecting condition.

Region		Expecting				Not Expecting			
		T-stat	x	y	z	T-stat	x	y	z
Fusiform Gyrus	L					4.55	-26	-56	-14
Inferior Temporal Gyrus	R					4.07	56	-54	-4
	L					3.58	-52	-68	2
Middle Occipital Gyrus	L					4.51	-46	-66	-6
Middle Temporal Gyrus	R					4.42	54	-60	2
Superior Parietal Lobule	R					4.8	26	-62	64
Cerebellum	L					3.83	-38	-66	-26

TABLE 8. High minus medium and low calorie contrast for 'expecting' and 'not expecting' conditions combined (all data). Listed peaks belong to significant clusters ($p < 0.05$, corrected for multiple comparisons).

Region		T-stat	x	y	z
Fusiform Gyrus	R	4.37	36	-70	-8
	L	4.33	-36	-62	-12
Inferior Occipital Gyrus	R	4.86	42	-76	-4
	L	3.79	-44	-76	-12
Inferior Temporal Gyrus	R	3.32	54	-54	-12
	L	5.32	-50	-72	-4
Middle Occipital Gyrus	R	3.53	54	-60	-6
	L	5.22	-48	-72	-6
Middle Temporal Gyrus	R	4.07	56	-52	-4
Superior Parietal Lobule	R	4.93	28	-60	64
	L	4.54	-32	-60	60

TABLE 9. Low minus high calorie contrast for 'expecting' and 'not expecting' conditions. Listed peaks belong to significant clusters ($p < 0.05$, corrected for multiple comparisons).

Region		Expecting				Not Expecting			
		T-stat	X	Y	Z	T-stat	X	Y	Z
Middle Temporal Gyrus (cluster 1)	R					4.97	56	-42	-2
Middle Temporal Gyrus (cluster 2)	R					5.01	64	-30	-16

DISCUSSION

The present study demonstrates that the neural response to food pictures is significantly modulated by expectancy to eat. In subjects who knew they could not eat, food cues significantly activated the DLPFC, anterior insula, hippocampus, parahippocampal gyrus and putamen. On the other hand, a region of caudal OFC that has been previously shown to respond to the appetitive properties of food and food cues (Gottfried et al., 2003; Kringelbach et al., 2003) was only recruited during the state of food expectation. The amygdala, mid-insula and fusiform gyrus were equally responsive in both conditions.

The brain regions activated in both states, namely the amygdala, insula, and visual areas, likely support the appetitive response to food cue presentation, independently of expectancy. Our subjects were hungry in both conditions, and the observed amygdala activation is consistent with earlier human research that has shown a hunger-induced amygdala response to food pictures (LaBar et al., 2001; Morris and Dolan, 2001). This response is positively correlated with recognition memory for food images (Morris and Dolan, 2001), emphasizing its role in motivated behaviors. Without manipulating the motivational state of subjects, Killgore et al (2003) observed amygdala activation to both high and low calorie food images, implying that this area may be reactive to a range of biologically relevant stimuli. Importantly, not only is the amygdala activated by food presentation but also by both pleasant and unpleasant tastes (O'Doherty et al., 2001) as well as by the anticipation of a pleasant taste (O'Doherty et al.,

2002). Furthermore, highly appealing menus tailored to participant's preferences also elicited a strong amygdala response (Arana et al., 2003; Hinton et al., 2004). It has been speculated that the amygdala along with the OFC (discussed below) signals the current incentive value of stimuli in a motivationally-dependent fashion (Rolls, 1994; LaBar et al., 2001; Morris and Dolan, 2001; Small et al., 2001; Baxter and Murray, 2002; Hinton et al., 2004; Holland and Gallagher, 2004). While primate research suggests that this may be a function of the basolateral amygdala (Baxter and Murray, 2002), this has yet to be substantiated in humans. The spatial resolution of fMRI as used here does not allow us to identify the specific amygdala nucleus involved.

The mid to anterior insula, a paralimbic structure positioned beneath the frontal operculum within the lateral sulcus, is often termed the 'ingestive cortex' due to its numerous feeding-related functions (Scott and Plata-Salaman, 1999). In this study, the left mid-insula responded to food pictures in both 'expecting' and 'not expecting' conditions. As for the amygdala, hunger-mediated activation of the insula is well-described (Tataranni et al., 1999; Morris and Dolan, 2001; Small et al., 2001; Hinton et al., 2004; Wang et al., 2004; St-Onge et al., 2005; Porubska et al., 2006), although insula activation to food stimuli while in the fed state has also been shown (LaBar et al., 2001). The insula receives inputs from taste, olfactory, and visual cortex (Scott and Plata-Salaman, 1999), and interoceptive information from the gut (Craig, 2002). It is proposed to integrate information about food cues and interoceptive state and transmit this information to other limbic

structures with which it is connected, such as the OFC, amygdala and ventral striatum (Augustine, 1996). Thus, apart from monitoring interoceptive cues, other proposed insular functions include taste sensation (Faurion et al., 1998; Pritchard et al., 1999; DelParigi et al., 2005), the processing of the reward value of food (Balleine and Dickinson, 2000; Small et al., 2001), incentive memory for food cues (Balleine and Dickinson, 2000) and food craving (Pelchat et al., 2004). Overall, our data support the hypothesis that the insula and visual areas are integral components of *a food knowledge circuit*, which is activated in response to visual food cues (Simmons et al., 2005), irrespective of expectancy to eat. While not explicitly tested in this experiment, our results and previous work suggest that neural responses in these regions are amplified during hunger (Tataranni et al., 1999; LaBar et al., 2001; Hinton et al., 2004; Malik et al., 2008), implicating them in the homeostatic control of feeding.

In the present investigation, the left caudal OFC response to food pictures was significantly greater when these were viewed in a state of expectancy to eat. Peak t-values for the left OFC were near coordinates previously shown to respond to food pictures (Simmons et al., 2005; Porubska et al., 2006) and to cues predicting the receipt of a pleasant taste (O'Doherty et al., 2002). This finding is consistent with primate electrophysiology data showing that neurons in this region respond to the presentation of a visual cue that predicts the delivery of a reward (Thorpe et al., 1983). Human studies indicate that this part of the OFC encodes the real-time reward value of a reinforcer. Specifically, fMRI studies employing variations of

the selective satiety paradigm reveal that the OFC, along with the amygdala, tracks changes in hedonic value such that neural activity decreases as a food (or associated cue) is devalued by satiation (Small et al., 2001; Gottfried et al., 2003; Kringelbach et al., 2003). In other words, the OFC likely enables switching of behavioral strategies as reinforcement contingencies change. Importantly, while both the amygdala and OFC participate in hedonic appraisal, the OFC itself appears to be involved when incentive value is used for action selection. This supports a partial functional divergence between the two structures (Arana et al., 2003; Hinton et al., 2004).

In the 'not expecting' state, subjects knew that they would not eat for at least one hour after the fMRI scan. Neural activity specific to this condition was localized in the DLPFC, anterior insula, hippocampus, parahippocampal gyrus (PHG) and dorsal striatum. Human imaging experiments with a feeding focus have formerly shown recruitment of these structures. While the hippocampus appears to be activated following food deprivation (Tataranni et al., 1999; St-Onge et al., 2005), the PHG data are conflicting. Small et al (2001) observed increasing PHG activity as chocolate was consumed to satiety whereas Labar et al (2001) reported the exact opposite response to food pictures (ie. heightened response during hunger which was abolished during satiety). One possible explanation for activation of these structures solely in the 'not expecting' scan is enhanced (food) craving in this state. Food-craving related changes have previously been reported in the hippocampus, insula and dorsal striatum (Pelchat et al., 2004), and drug

craving reportedly involves the aforementioned structures as well (Grant et al., 1996; Breiter et al., 1997). In the present study however, a differential response in the subjective craving scores between the 'expecting' and 'not expecting' conditions was not observed, possibly due to a limitation in our Likert-type scale. Participants rated their hunger and craving levels on an integer scale ranging from one to five. As subjects had been food-deprived at baseline, maximum scores were often recorded at all time points in both conditions, yielding a ceiling effect.

The DLPFC (middle frontal gyrus, Brodmann area 46) was also significantly activated in the 'not expecting' state. The DLPFC is involved in a host of executive cognitive tasks, especially when planning of behavior is required (Dagher et al., 1999; van den Heuvel et al., 2003). PET imaging studies have additionally implicated this area in the termination of feeding, as regional cerebral blood flow is increased following both liquid meal consumption (Tataranni et al., 1999) and chocolate-induced satiety (Small et al., 2001). In fact, blunted postprandial DLPFC activity in obese males (Le et al., 2006), and hyperfunction of functionally-related ventromedial PFC in Prader-Willi patients (Holsen et al., 2006; Miller et al., 2007), suggest a role of the prefrontal cortex in the suppression of appetite and food intake. Furthermore, the DLPFC appears to be selectively responsive to calorie-dense images (Killgore et al., 2003) as well as to taste stimuli (Kringelbach et al., 2004), although the latter may reflect an effect of taste on cognitive or attentional processing. In particular, the left DLPFC does not appear to be involved in direct reward-related activity, as there was no correlation

between taste pleasantness and DLPFC activation (Kringelbach et al., 2004). In our study, left DLPFC activation was exclusively seen in the 'not expecting' state. We hypothesize that within the context of this experiment, the cognitive control network, particularly the DLPFC, was engaged to suppress appetite. As subjects knew they could not eat for an extended period of time, inhibitory control in response to food cue exposure was probably required. Since response inhibition is known to critically involve the DLPFC (Blasi et al., 2006), this area may mediate top-down processing of external stimuli contingent on whether the reward is seen as attainable. Thus, the DLPFC may integrate information about the motivational state, expectancy and reward cues and use this information in the regulation and planning of food seeking/avoiding behavior. In contrast to our current findings, a similar study with smoking cues found that the DLPFC was recruited only in the 'expecting' state (McBride et al., 2006). Worth mentioning however is the fact that the DLPFC location activated in the current study differs from the area activated in two expectation studies involving cigarette cues (Wilson et al., 2005; McBride et al., 2006).

The majority of the observed activation in our subjects was unilateral, preferentially on the left (Table 1). While certain parts of the insula exhibited bilateral activation, it was sub-threshold on the right. This may be due to insufficient statistical power such that increasing the sample size could yield bilateral responses; however for the other structures, the activation was clearly left-lateralized. While an asymmetrical model of the neural control of normal

feeding behavior has not yet been proposed, at least two imaging studies corroborate our findings (Del Parigi et al., 2002; Porubska et al., 2006). Employing a comparable experimental protocol with food and non-food images, Porubska et al (2006) observed predominantly left-sided food-related activation of the insula and OFC in fasted participants. Similarly, the left insula/ frontal operculum and piriform cortex displayed intense activity in extremely hungry individuals administered a small amount of liquid formula (Del Parigi et al., 2002). This raises the possibility that feeding is hemispherically asymmetric.

Finally, since only males were tested in this study, a similar experiment should be conducted in females as gender differences in the neural response to food stimuli have been reported (Uher et al., 2006)

In summary, all of the regions significantly activated in this investigation have previously been implicated in food reward. Interestingly however, the present study demonstrates that the structures recruited during the 'expecting' state were in part dissociable from those in the 'not expecting' state, with overlap in the amygdala and insula. The amygdala and insula activity may represent a general appetitive response to food inputs whereas separate areas of the frontal cortex may be engaged depending on the cognitive state; more specifically, the DLPFC for the suppression of appetite in the 'not expecting' condition, and the OFC for the incentive-guided response in the 'expecting' condition. Further work is necessary to determine how the state of expectancy modulates neural response to

food cues as well as how the prefrontal motivational systems regulate consummatory behavior.

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MODULE III:

General Discussion

PREFACE MODULE III

Module III, the last section of this thesis, presents the general discussion. Upon summarizing the major results, the single chapter (Chapter 6) addresses a number of issues, which for the most part, are relevant to both experiments. Since both investigations employed functional Magnetic Resonance Imaging with visual food stimuli, as well as entailed the modulation of intrinsic motivational state (albeit using different measures), it follows that there is considerable overlap between the studies.

6

PERSPECTIVES

6.0 Overview

Functional Magnetic Resonance Imaging (fMRI), an approach that permits exploration of patterns of brain activation associated with various mental processes, has yielded important empirical findings regarding the multidimensional aspects of feeding behavior. Employing this technique, experiments reported in this dissertation have mapped the neural response to food cues following manipulation of ghrelin concentrations (Study 1) and state of food expectation (Study 2). Main findings include the following: 1) ghrelin administration produced widespread activation of limbic, paralimbic and visual circuitries, networks implicated in reward, motivation, memory and attention, 2) ghrelin's effects on the amygdala and OFC response were correlated with subjective hunger scores, 3) intravenous ghrelin afforded a memory advantage for

appetitive cues, 4) cerebral structures engaged during the 'expectant' and 'not expectant' states showed both convergence (amygdala and mid-insula) and dissociation, and 5) state-dependent brain activity was observed in the dorsolateral prefrontal cortex, anterior insula, hippocampus, parahippocampal gyrus, putamen and caudal OFC, with all but the latter exclusively reactive during the 'not expectant' condition. In addition, these investigations confirm the use of food pictures as potent appetitive triggers.

With respect to these findings, this final chapter will address the following topics: a) is there a physiological relationship between the two research studies, b) are these experimental data consistent with the prevailing model of feeding behavior, c) what are the shortcomings of functional imaging investigations, d) what are the public health implications of this work, and e) what lies ahead for feeding-related research.

6.1 Linking the Experiments

Numerous studies have demonstrated that the hungry brain's response to food cues is enhanced both in terms of regions recruited and amount of BOLD signal, and markedly differs from the satiated state (Tataranni et al., 1999; LaBar et al., 2001; St-Onge et al., 2005; Porubska et al., 2006; Führer et al., 2008). We have described for the first time that *exogenous* administration of the appetite-stimulating hormone ghrelin, rapidly and potently transformed a metabolically 'neutral' central nervous system into a 'hungry' one; and accordingly, activated

multiple appetitive structures including the amygdala, insula, hippocampus, orbitofrontal cortex, midbrain, dorsal striatum, and pulvinar (Study 1). From a theoretical perspective, fasting-induced increases in *endogenous* ghrelin levels (Study 2) should have similar effects on brain activation to the effects seen after *exogenous* ghrelin administration (Study 1). In this regard, collapsing the 'expectant' and 'not expectant' data acquired in our second study (Chapter 5) facilitated the assessment of brain activity in energy-deprived subjects, who presumably had elevated circulating ghrelin levels (Cummings et al., 2001; Tschop et al., 2001; Cummings et al., 2004). Indeed, the food minus scenery t-map generated in this analysis was comparable to or superimposable on, that computed in the ghrelin condition of the first experiment (Figure 6-1).

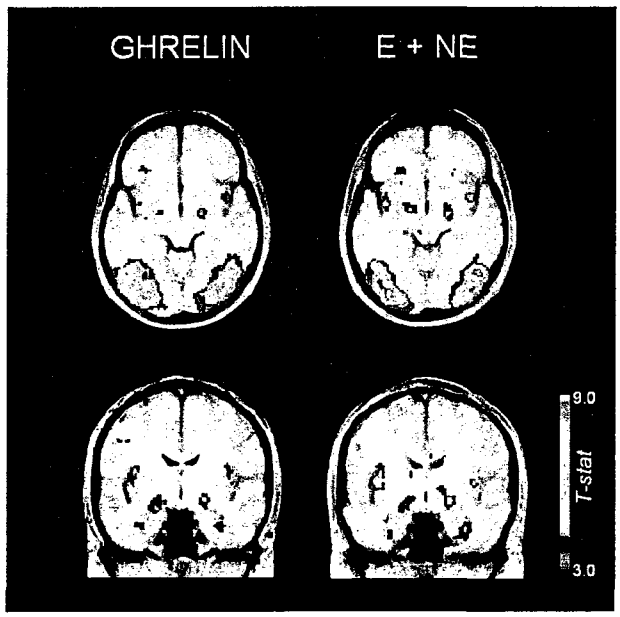

Figure 6-1. Food minus Scenery T-maps for the Two Research Studies. The ghrelin images represent the mean of 12 subjects, following treatment with ghrelin. The images on the right are the combined average of the 10 'expectant' (E) plus the 10 'not expectant' (NE) scans. Horizontal and verticofrontal slices are presented (MNI coordinates: 20, -10, -8).

Areas such as the amygdala (bilateral), mid-insula (bilateral), OFC (L) and pulvinar (L), were significantly responsive to palatable food pictures, in both ghrelin and expectation studies. Further, two-tailed t-tests showed no differences between regional BOLD signal intensities when comparing the two experiments. Inability to detect differences may be a consequence of insufficient statistical power in the analysis process (Study 1: n=12 scans; Study 2: n=20 scans). Nonetheless, we speculate that endogenous ghrelin concentrations achieved after approximately eight hours of fasting (Study 2) were equivalent to (or approached) those attained following a 1 μg/kg ghrelin injection 3 hours post-breakfast (Study 1). This would imply that the aforementioned areas are at least in part responding to ghrelin (endogenous or exogenous), to augment the hedonic value or motivational salience of food-related cues. Such functional roles have previously been assigned to these cortical and sub-cortical areas (Module I).

6.2 Modeling Feeding Behavior

Most widely accepted, is the two-tier model of feeding. It posits that ingestive behavior is regulated by both bottom-up (homeostatic) and top-down (non-homeostatic) processes. As described in Chapter 1, the homeostatic side focuses on the effects of peripheral signals on hypothalamic peptidergic pathways guiding nutrient intake, while the non-homeostatic side participates in the incentive, hedonic and cognitive control of eating via a network of alternate regions. The two-tier structure additionally proposes that non-homeostatic factors may override homeostatic controls. This framework is further expanded by recent

animal research and human brain imaging studies which demonstrate that the metabolic products (i.e. leptin, PYY and ghrelin) that modulate hypothalamic activity, also affect brain regions traditionally associated with reward and motivation such as the OFC and the striatum (Naleid et al., 2005; Abizaid et al., 2006b; Baicy et al., 2007; Batterham et al., 2007; Farooqi et al., 2007). The data presented in this thesis supplement these findings and favor an interaction between the two branches of energy balance. In Experiment 1, appetizing food cues failed to elicit activation of the rewarding brain as assessed by fMRI, until ghrelin, a homeostatic signal, was intravenously dispensed to study participants. Further, the state-independent neural response to food stimuli in Experiment 2 paralleled that observed in the first investigation. Therefore, it appears that motivational state (dependent on intrinsic factors) has the ability to alter how rewarding or palatable a food item may be perceived. Though hypothalamic activity was absent in both of our studies (a reasonable outcome given that hypothalamic neurons don't consistently respond to visual information in event-related protocols), this area has previously been shown to respond to visual food cues (Cornier et al., 2007); again, reinforcing an interaction between external non-homeostatic sensory inputs and homeostatic circuitry. In sum, the current data show that cross-talk between homeostatic and non-homeostatic processes, is unmistakably evident. This suggests that both limbs of the prevailing model of feeding behavior are inter-reliant and complimentary.

6.3 Methodological Considerations

Feeding-related functional Magnetic Resonance Imaging (fMRI) studies, including those presented in this thesis, suffer from several limitations. One key challenge is successfully imaging appetitive regions such as the orbitofrontal cortex (OFC) and the hypothalamus. The OFC's proximity to the air-filled sinuses boosts the probability of geometric distortion, signal intensity dropout and susceptibility artifacts. Similarly, the hypothalamic structure, positioned deep in the midbrain, is difficult to image. Although activity in the hypothalamus has previously been documented with PET and fMRI techniques (Matsuda et al., 1999; Tataranni et al., 1999; Gautier et al., 2000; Liu et al., 2000; O'Doherty et al., 2002; Smeets et al., 2005; Cornier et al., 2007), the small size of this region frequently impedes signal detection. In effect, confines in spatial resolution reduce the power to identify responses in this site. The inability to differentiate regional sub-divisions or composite nuclei (e.g. in the amygdala) is also a methodological limitation.

Duration of scans is another limiting factor. With increasing length, movement artifact and habituation effects become significant concerns. Presently, fMRI procedures span approximately 60 minutes (45 minutes of functional imaging plus a 15 minute structural acquisition); hence, a sound investigation must be fitted to this time constraint.

Next, technical confounds restrain experimental design in the scanner. Food-related visual, olfactory, and gustatory stimuli can be, and have been presented in the scanning machine (O'Doherty et al., 2000; LaBar et al., 2001; O'Doherty et al., 2001; O'Doherty et al., 2002; Gottfried et al., 2003; Simmons et al., 2005; St-Onge et al., 2005; Porubska et al., 2006). However, an ideal examination of energy balance entails imaging the actual ingestive component (i.e. a meal episode), which is currently not feasible in the magnet. In essence, the scanner environment is artificial and not characteristic of a typical feeding experience, irrespective of the protocol employed

6.4 Implications of Research

Exogenous ghrelin's capacity to stimulate hunger, enhance the appeal of food cues and stimulate memory operations (Study 1), are interesting findings with therapeutic potential. Preliminary clinical investigations have demonstrated the efficacy of ghrelin agonism in treating chronic illness-related cachexia (Ashby et al., 2008) while animal experiments have yielded positive outcomes for ghrelin therapy in the anorexia of aging (Ariyasu et al., 2008). Encouraging evidence has also surfaced for the use of ghrelin in treating Alzheimer's-like cognitive impairments in mice (Diano et al., 2006).

In contrast to ghrelin supplementation, a novel area of pursuit is the development of treatments that block ghrelin activity. Anti-ghrelin agents are aspired to help counter obesity, a ghrelin-sensitive condition (Druce et al., 2005)

via a modulatory effect on appetite and energy intake. Indeed, *ghrelin receptor antagonists* have been demonstrated to acutely reduce food intake in lean and obese rodents (Asakawa et al., 2003; Beck et al., 2004), and *anti-ghrelin Spiegelmers*, which are synthetic oligonucleotides that specifically bind octanoylated ghrelin and prevent receptor-peptide interactions, also significantly suppress the orexigenic effects of peripherally administered ghrelin in rats (i.e. they inhibit ghrelin-induced food intake) (Helmling et al., 2004; Kobelt et al., 2006). Further, Holst and colleagues have recently suggested that inhibition of the constitutively active ghrelin receptor with an *inverse agonist,* may reduce the inter-meal signaling "set point" thereby enhancing sensitivity to metabolic satiety signals such as PYY and leptin and eradicating snacking behavior (Holst et al., 2003; Holst and Schwartz, 2004). In sum, antagonists, spiegelmers and inverse agonists, are anticipated treatment options for human obesity as they are projected to decrease nutrient consumption.

Despite their promise, ghrelin-based pharmacological therapies must be approached with caution. As discussed in Chapter 2 and Thesis Study I (Chapter 4), ghrelin mediates multiple physiological functions, such as *feeding, memory, reward*, and *cardiovascular* functions. The widespread distribution of ghrelin receptors and ghrelin-positive axon terminals across the central nervous system, and in particular in the hypothalamus, hippocampus, amygdala, VTA, ventral striatum, and dorsal raphe (Guan et al., 1997; Cowley et al., 2003; Zigman et al., 2006), supports many of the aforementioned functions and additionally favors

roles for the peptide in the regulation of *emotion* and/or *mood*. Modulation of ghrelin concentrations may therefore impair one or more of ghrelin's biological activities. Indeed, a substantial excess of deaths owing to coronary heart disease and suicide have been reported after bariatric surgery (Omalu et al., 2007), weight-reducing procedures often associated with decreases in endogenous ghrelin levels (Cummings et al., 2002). It is worth mentioning that an elevated incidence of adverse psychiatric events (i.e. depressive mood disorders) have also been observed following treatment with Rimonabant (Christensen et al., 2007; Isoldi and Aronne, 2008), an anti-obesity drug which antagonizes cannabinoid CB1 receptors distributed in the same neural circuitry as ghrelin receptors (Rinaldi-Carmona et al., 2004; Di Marzo and Matias, 2005; Le Foll and Goldberg, 2005; Bellocchio et al., 2006; Pagotto et al., 2006; Mangieri and Piomelli, 2007). These findings suggest that interference in ghrelin (and cannabinoid) signaling may promote weight loss at least in part by reducing the hedonic value or satisfaction of eating; yet, such signaling disruptions in the reward and mood pathways may also decrease the pleasure of other experiences thereby triggering negative affect. Keeping in mind that obesity is itself associated with depression (Dixon et al., 2003; Onyike et al., 2003; Bloom et al., 2008), thorough clinical trials must evaluate the risk to benefit ratios of medication targeting the ghrelin system.

6.5 Future Research

Multiple directions for future human brain mapping research have emerged as a consequence of the results of this dissertation. First, replication of these experiments employing [^{11}C] raclopride positron emission tomography (PET) is a logical follow-up. This method, which pairs brain imaging (PET) with radioligand binding ([^{11}C] raclopride), facilitates region-specific assessments of dopamine release and neuronal activation. In theory, pharmacological or cognitive challenges that augment endogenous dopamine levels enhance the occupancy of dopamine D2 receptors by dopamine, and therefore reduce receptor availability for raclopride (a D2 receptor antagonist) binding. Thus, changes in binding potential (calculated using PET analysis) provide a quantitative index of synaptic dopamine release. Since some of the neural regions responsive in the current fMRI studies belong to dopamine pathways, repeating these experiments using the [^{11}C]raclopride/PET technique will enable an examination of chemical neurotransmission in vivo (as the BOLD technique used in this thesis is not in itself sensitive to changes in dopamine release). To elaborate, such investigations will elucidate whether different states of expectation, or exogenous ghrelin administration (combined with visual food cues), modulate dopamine neuron activity within the mesolimbic reward circuitry, in humans. Surely, both of these factors have been demonstrated to alter dopamine systems in animals (Duvauchelle et al., 2000b; Duvauchelle et al., 2000a; Abizaid et al., 2006b).

Second, it will be beneficial to examine different populations. Gender differences should be gauged by repeating these studies in women. Additionally, clinical groups who suffer compromised feeding behavior such as those afflicted with eating disorders or obesity would also be interesting study candidates. In fact, conducting analogous studies in clinical cohorts, pre- and post- intervention strategies, could be quite informative. Tracing the neural responses in this manner may impart greater knowledge regarding the mechanisms of normal feeding regulation as well as the etiology of metabolic diseased states.

Third, imaging *ghrelin's effects* is an innovative domain, with numerous possibilities. Using fMRI with visual food cues, we demonstrated that this peptide potently stimulates central orexigenic circuitry. Prospective fMRI investigations must evaluate ghrelin's response to other forms of food-related sensory stimulation, including olfaction and gustation, which are primary reinforcers of food intake. Essentially, the implementation of multi-sensory paradigms is a desirable tactic as it mimics a more holistic feeding experience. Moreover, due to the extensive distribution of ghrelin receptors and processes across the meso-limbic network, ghrelin's role in addiction (Cummings et al., 2007), memory and emotion, are exciting areas of pursuit.

Finally, the next generation of imaging research is directed towards higher field strength magnets. Currently, the vast majority of feeding-associated fMRI investigations, including those presented in this work, have been conducted on 1.5

T MR systems (LaBar et al., 2001; Killgore et al., 2003; St-Onge et al., 2005; Killgore and Yurgelun-Todd, 2006; Porubska et al., 2006; Smeets et al., 2006; Uher et al., 2006; Farooqi et al., 2007). However, more and more research facilities are opting to replace the 1.5 T scanners for those operating at 3.0 T. The chief advantage of 3.0 T MRI over the standard field strength of 1.5 T is that it roughly doubles the signal to noise ratio. As a result, 3.0 T systems generate higher spatial resolution scans that enable the visualization of more detailed anatomical structures (eg. sub-nuclei). Further, higher field strength systems enhance sensitivity to contrasts of interest including functional BOLD effect (Voss et al., 2006; Scarabino et al., 2007; Soher et al., 2007). Future work on 3.0 T scanners and even stronger ones ($\geq$ 4.0 T) may therefore provide additional insights regarding the neural substrates and networks that are modulated by ghrelin and/or the state of expectation. Stronger magnets may not only heighten regional signals in areas already known to be activated with the weaker 1.5 T systems; yet, may also allow detection of additional structures and/or sub-nuclei not apparent with 1.5 T imaging.

6.6 Summary and Conclusion

In summary, the work presented in this dissertation has significantly contributed to the field of feeding behavior. The main experimental results, drawn from fMRI studies, advocate that the cerebral response to visual food stimuli in humans is significantly modulated by the orexigenic brain-gut peptide ghrelin, and also by the state of food expectation. Specifically, we show that the

'expecting' and 'not expecting' food reward conditions engaged both common (amygdala and mid-insula) and distinct ('not expecting': DLPFC, hippocampus, putamen, right anterior insula; 'expecting': OFC) neural substrates (Study 2), and that intravenous ghrelin administration modulated the neural response to food pictures in brain areas involved in reward processing, motivation, memory, and attention; namely the amygdala, insula, OFC, hippocampus, striatum, pulvinar and fusiform gyrus (Study 1). The latter indicates that homeostatic feeding signals such as ghrelin may promote food consumption by enhancing the appetitive response to food-related cues. In addition, the current projects add to the limited pool of brain imaging studies that have employed *visual* food stimulation to investigate ingestive behavior, and reveal that the neural response to food cues in humans is influenced by *both* intrinsic factors related to the hunger and satiety cascade, such as ghrelin, and extrinsic cognitive factors such as reward expectation.

To conclude, feeding is a complex behavior that has yet to be completely resolved. Nonetheless, the last decade has made significant advances in identifying the brain regions and metabolic signals that control 'homeostatic' feeding, and it is aspired that the years to come will clarify the central substrates and mechanisms underlying 'extra (or non)-homeostatic' feeding regulation. As well, the future promises to further unveil how the rewarding, cognitive and emotional brain interacts with so-called 'homeostatic' pathways (and/or vice versa) to regulate food intake and nutrient balance. Indeed, both animal models

and refined human brain imaging techniques will serve as crucial research tools for the investigation of ingestive processes. Ultimately, a greater understanding of human feeding behavior will have important implications for the treatment and management of obesity, a rising concern across the globe.

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APPENDIX

Study 1

SUPLEMENTARY MATERIALS

APPENDIX 1: SUPPLEMENTARY EXPERIMENT

These additional experimental data, in a separate group of subjects, are presented in support of our main findings.

Subjects

Twenty healthy, right-handed, and non-obese (Body Mass Index ± SEM, 22.8 ± 0.4 kg/m^2) male subjects between the ages of 18-35 (mean age ± SEM, 25 ± 1.3) were recruited by advertisements. All participants were medication-free and had normal or corrected-to-normal vision. Exclusion criteria were identical to those used in the first experiment. Abnormal eating patterns and behavior were excluded via the Dutch Eating Behavior Questionnaire (Van Strien et al., 1986) and the Three-Factor Eating Questionnaire (Stunkard et al., 1985). The research protocol was approved by the Montreal Neurological Institute Research Ethics Board, as well as the Therapeutic Products Directorate of the Canadian government.

Materials

Pharmaceutical-grade human ghrelin was purchased from Clinalfa (Laeulfingen, Switzerland). The visual stimuli were full-color photographs of foods, scenery, and facial expressions. Food and scenery photographs were similar to those used in the first experiment. Photographs of faces depicted a disgusted, fearful, happy, or neutral expression. These photographs were chosen from the Karolinska Directed Emotional Faces database. Data from the facial expression pictures is not discussed here.

Experimental Procedure

There were two fMRI sessions approximately one week apart. Prior to one scan, subjects received a bolus injection of ghrelin (1.0 μg/kg over 1 minute) in normal saline and, during the other, normal saline alone, in single-blinded fashion. These two conditions were counterbalanced across subjects to avoid any possible order effects. In order to minimize variability due to circadian rhythms, each scan occurred at the same time of day for each subject (i.e. from 11am-12:30pm or 12:30pm-2pm).

After a 12 hour overnight fast, ghrelin or saline administration occurred approximately three hours after an investigator-provided standard test breakfast (as in the first experiment). Subjects answered questions pertaining to their hunger, nausea, boredom and irritability levels on a visual analog scale (e.g. how hungry are you right now?).

Stimulation Paradigms

A total of 72 different images were presented during each of the three functional sequences of which 24 were food images, 24 were scenery images, and 24 were images of faces displaying disgusted, fearful, happy or neutral facial expressions.

The order in which the stimuli were viewed was randomized for all subjects. Each image was presented for 3 seconds with an average interstimulus interval (ITI) of 4 seconds. Longer ITIs ("null events") were also included to gain an estimate of baseline activity. In an effort to increase the effective sampling rate and allow an even sampling of voxels across the entire brain volume the presentation of the stimuli was slightly de-synchronized with regards to the onset of volume acquisitions. Stimuli were presented on a projector screen using E-PRIME software (Psychology Software Tools, Pittsburgh PA).

On the next day, subjects returned to the lab for a memory task. They viewed 36 of the previously seen photographs randomly intermixed with 36 novel photographs. Subjects indicated whether each photograph had been seen previously or not.

fMRI Acquisition

FMRI scanning was performed at the Montreal Neurological Institute using a 3T Siemens Trio with a standard head coil. Functional scanning began 30 minutes after ghrelin or saline administration. One anatomical scan and three functional sequences comprised each scanning session. Following the anatomical scan, functional T2* weighted images were acquired using blood oxygenation level-dependent (BOLD) contrast, (T_R= 2500 msec, T_E= 30ms, flip angle = 90°, FOV = 256mm, voxel size 4 x 4 x 4 mm^3) covering the entire brain (34 interleaved slices at an angulation of -30 ° to the anterior-posterior commissural plane). Two dummy images were taken at the start of each functional scan to reduce non-steady state effects.

fMRI Data Analysis

Images from each scan were realigned with the third frame of the first run, motion corrected, and smoothed using an 6 mm full-width half-maximum isotropic Gaussian kernel. A general linear model was designed using separate regressors for food, scenery and face pictures, composed of boxcar functions convolved with a standard hemodynamic response function. Contrasts of food minus scenery were calculated to generate t-maps. Parametric images were transformed into standard stereotaxic space (Talaraich and Tournoux, 1988) derived from the MNI 305 template (Collins et al., 1994) and a mixed effects statistical model was used to subsequently conduct a group analysis (Worsley et al., 2002). In order to compare the ghrelin and saline conditions, the effect sizes from the general linear model were extracted from the peak voxels of areas of significant activation. To avoid biasing the analysis, the maximum peak location for each region was selected from the ghrelin and saline t-maps, and effect sizes extracted from the ghrelin and saline analyses using the corresponding coordinates.

Results

Only the results of the food minus scenery contrast are presented here. Table S1 and Fig. S1 demonstrate that ghrelin increased the brain response to food pictures (when compared to scenery) in the bilateral insula, left amygdala, hippocampus

and parahippocampal gyrus (PHG), and bilateral fusiform and pulvinar, among other visual areas. All effects were highly significant at $p < 0.0005$. The BOLD effect in the left amygdala and bilateral insula correlated with hunger ratings (Fig. S2). Memory for food pictures (tested the next day) was significantly greater for the ghrelin session than the saline session ($p < 0.001$), and the memory score correlated with the increased BOLD effect size due to ghrelin (i.e. ghrelin minus saline sessions) in the left amygdala.

TABLE S1: Food minus scenery contrast for the ghrelin and saline states
Brain areas of significant BOLD signal contrasts between food and scenery cues under the ghrelin and saline states for all subjects. All peaks are listed at $p<0.001$ uncorrected with a minimum cluster extent of 20 contiguous voxels.

Region		Ghrelin				Saline			
		x	y	z	t-stat	x	y	z	t-stat
Orbitofrontal Cortex	L	-24	32	-12	4.36	-22	30	-14	5.20
Middle Frontal gyrus	L	-44	22	26	4.38	-46	26	20	4.88
Precentral gyrus	R	46	2	28	5.43	50	4	30	5.86
Insula	R	36	4	-10	6.79	38	4	-8	4.41
	L	-38	0	-4	6.43	-36	-6	-8	6.21
Parahippocampal gyrus	L	-32	-32	-24	5.84	-32	-6	-28	5.15
Postcentral gyrus	L	-58	-20	26	4.42	-62	-22	32	5.16
Posterior Cingulate gyrus	L	-2	-32	30	4.99				
Inferior Parietal lobule	L	-44	-40	46	6.01	-46	-38	42	5.51
Fusiform gyrus	R	34	-48	-18	7.11	34	-56	-18	6.80
	L	-34	-56	-14	9.18	-36	-56	-14	9.75
Superior Parietal lobule	R	30	-58	56	6.63	28	-58	50	5.77
	L	-26	-62	54	6.82	-24	-60	48	6.81
Middle Occipital gyrus	R	44	-68	-10	10.25	40	-76	-8	10.41
	L	-40	-74	-8	11.43	-42	-72	-8	11.68
Inferior Occipital gyrus	R	38	-84	0	9.44	42	-82	-4	10.41
Lingual gyrus	L	-26	-86	-8	9.71	-24	-86	-4	8.09
Cuneus	R	16	-94	0	8.78	18	-94	2	8.63
	L	-18	-100	-4	6.86	-14	-98	2	7.32
Amygdala	L	-20	-10	-8	5.23				
Hippocampus	L	-34	-20	-22	4.68				
Pulvinar	L	-20	-30	0	5.96	-20	-30	0	5.20
	R	20	-30	-4	4.76				

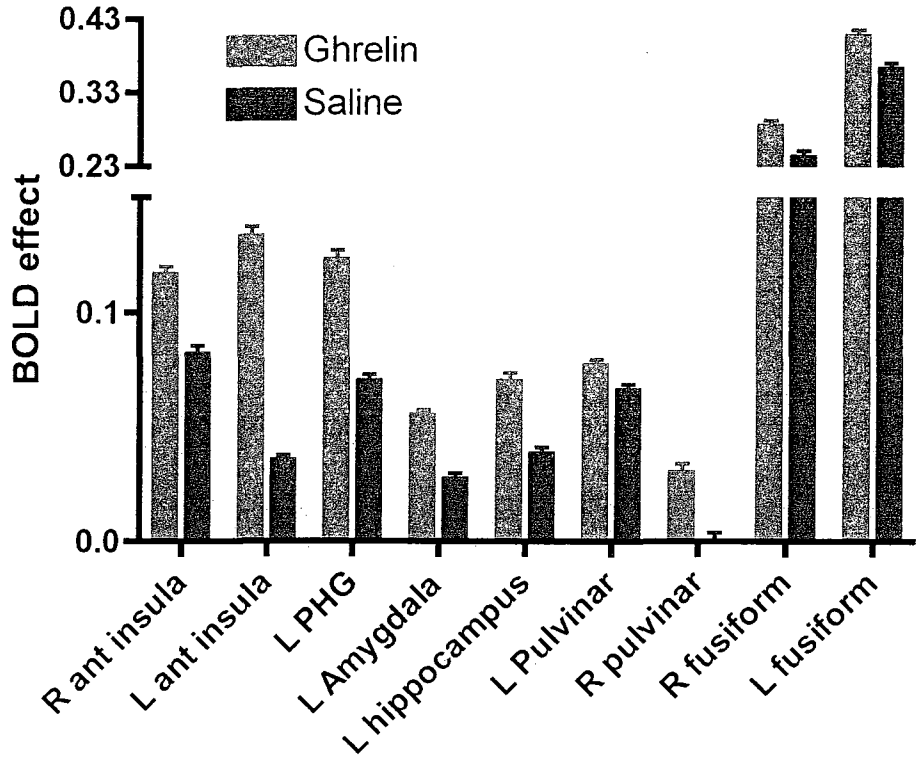

Fig. S1 Effect of ghrelin on food minus scenery contrast. BOLD effect refers to the effect size of the contrast food minus scenery. Error bars are the SD of the general linear model effect size. All $p < 0.0005$.

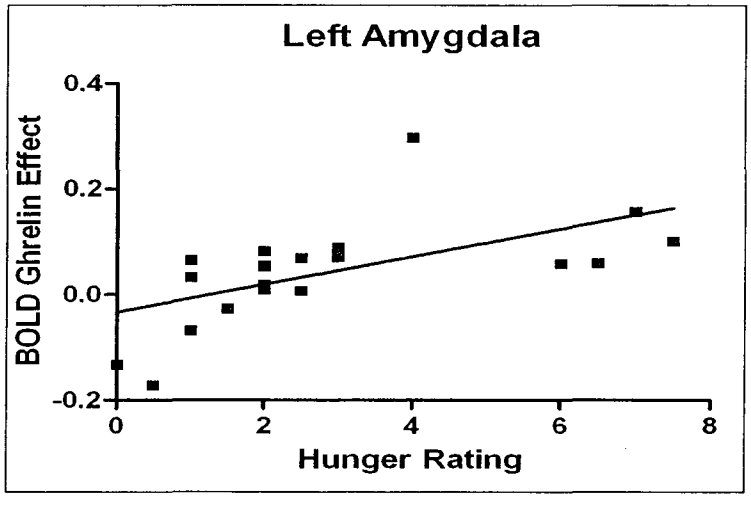

Fig. S2 Significant correlation between hunger rating and BOLD signal increase in the left amygdala (difference between ghrelin and saline scans).

APPENDIX 2: SUPPLEMENTARY MATERIAL

Table S2: Food minus scenery contrast (control 1 and control 2 combined) for the control-control group. (Regions belong to clusters significant at $p < 0.05$, corrected for multiple comparisons; * cluster not significant but chosen a priori)

		t-stat	x	y	z
Fusiform Gyrus	R	5.01	40	-68	-16
Fusiform Gyrus	L	4.58	-32	-56	-18
Inferior Frontal Gyrus	L	4.88	-52	8	26
Inferior Occipital Gyrus	R	4.66	48	-80	-4
Inferior Occipital Gyrus	L	7.5	-42	-74	-12
Inferior Parietal Lobule	L	4.81	-46	-38	48
Inferior Temporal Gyrus	R	4.12	40	-62	-2
Lingual Gyrus	R	3.36	16	-86	-12
Middle Occipital Gyrus	R	6.77	48	-64	-8
Superior Parietal Lobule	L	4.14	-30	-60	60
Insula*	L	4.48	-38	-8	8

Table S3: Interaction effect: areas with greater activation in the ghrelin-control than the control2-control1 condition. Contrast consisting of [(ghrelin – control) – (control2-control1)]. All peaks meeting a criterion of $p < 0.001$ ($T > 3.0$) uncorrected and identified in the previous t-map (Table 1, main manuscript) are listed. The right amygdala peak does not reach significance as defined here. There were no significant peaks in the reverse contrast.

		t-stat	x	y	z
OFC	L	3.05	-36	36	-2
Anterior Insula	L	3.17	-28	16	16
	R	3.39	34	8	18
Amygdala	L	3.09	-30	-4	-16
	R	2.98	22	-14	-12
Mid-Insula	L	3.21	-36	-14	14
SN/VTA	R	3.26	10	-26	-14
Pulvinar	L	3.74	-14	-38	6
Fusiform	R	3.17	44	-60	-20
	L	3.09	-18	-72	-14

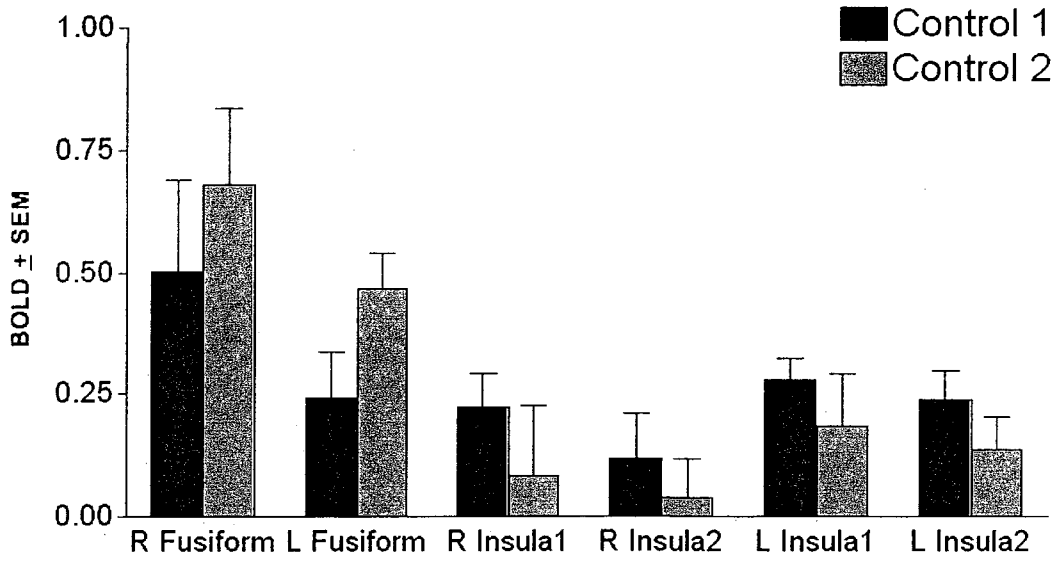

Figure S3 Control/Control group

BOLD effect (food minus scenery) during control 1 and control 2 at areas that showed an effect in the control-control study. Voxel coordinates that were used are peak values from the food minus scenery contrast for the two control blocks combined. Note that all insula activation was sub-threshold. There were no significant differences between the two conditions.

Coordinates used to construct bar graph.

Region	Hemisphere	x y z	t-stat
Fusiform	Right	(40 -68 -16)	5.01
	Left	(-32 -56 -18)	4.58
Insula	Right 1	(40 -8 8)	3.67
	Right 2	(40 -4 -2)	3.49
Insula	Left 1	(-38 -8 8)	4.48
	Left 2	(-40 -4 -6)	3.64

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HPLC Results

Ghrelin sample dissolved with double distilled water at time = 0 minutes

This solution placed at room temperature and passed through HPLC at different time points to determine short term stability (0-180 minutes).

Time point 1 = 0 minutes

- Solution prepared and the required volume injected into the HPLC system
- All subsequent runs were compared to this one
- Get 1 ghrelin peak at 15.8 min

Time point 2 = 45 minutes

- Get 1 ghrelin peak at 15.8 min and no peaks before ghrelin peak, therefore no cleavage of peptide and no degradation products

Time point 3 = 90 minutes

- No peaks before ghrelin
- Main ghrelin peak might be 2 peaks

Time point 4 = 135 minutes

- Main ghrelin peak equals 2 peaks

Time point 5 = 180 minutes

- Peak is lower yet in same place as other three (ran 50 µl sample instead of 80 µl)

CONCLUSION: Peptide stable up to 45 minutes

Other points:

- The retention time (Rt) is about 2 minutes more than the company because flow was decreased from 0.8 ml/min to 0.5 ml/min (current system cannot handle higher pressures)

- Inside column: 60% acetonitrile & 40% water
- Upon dissolving ghrelin with double distilled water, solution was clear and transparent (i.e powder completely dissolved)
- pH at the end of HPLC = 7 (determined using litmus paper)

HPLC Protocol

The HPLC conditions for Ghrelin C-S-142 are as follows:

Column: MN Nucleosil C18 250x3mm 300-5
Eluent A: 0.05 M TEAP pH 2.25
Eluent B: Eluent A + ACN (40:60)
Flow: 0.8 ml/min
Gradient: 30 - 60%B in 30 min
Detection: UV 215 nm
Temp: 25°C
Sample: vial dissolved in 500 µl Milli-Q-water (lyophilized powder must dissolve completely)
Inj. Vol.: 80 µl

Analyses to be done:

1. Appearance
2. pH of reconstituted ghrelin solution
3. HPLC at specific time points post-reconstitution (some or all of these times: 0, 15, 30, 45, 60, 90, 120, 180 minutes)

Supplies:

1. Nucleosil C18 (ODS) column
2. TEAP (Triethyl ammonium phosphate solution)
3. Ghrelin vials (100 µg)

*N.B. Merck does not filter reconstituted ghrelin solution

Run File : c:\star\data\hinko\5-11-04,ghrelin time-0.run
Method File : c:\star\30-60%b-30',fr-0.5, 215nm, teap.mth
Sample ID : Ghrelin time-0

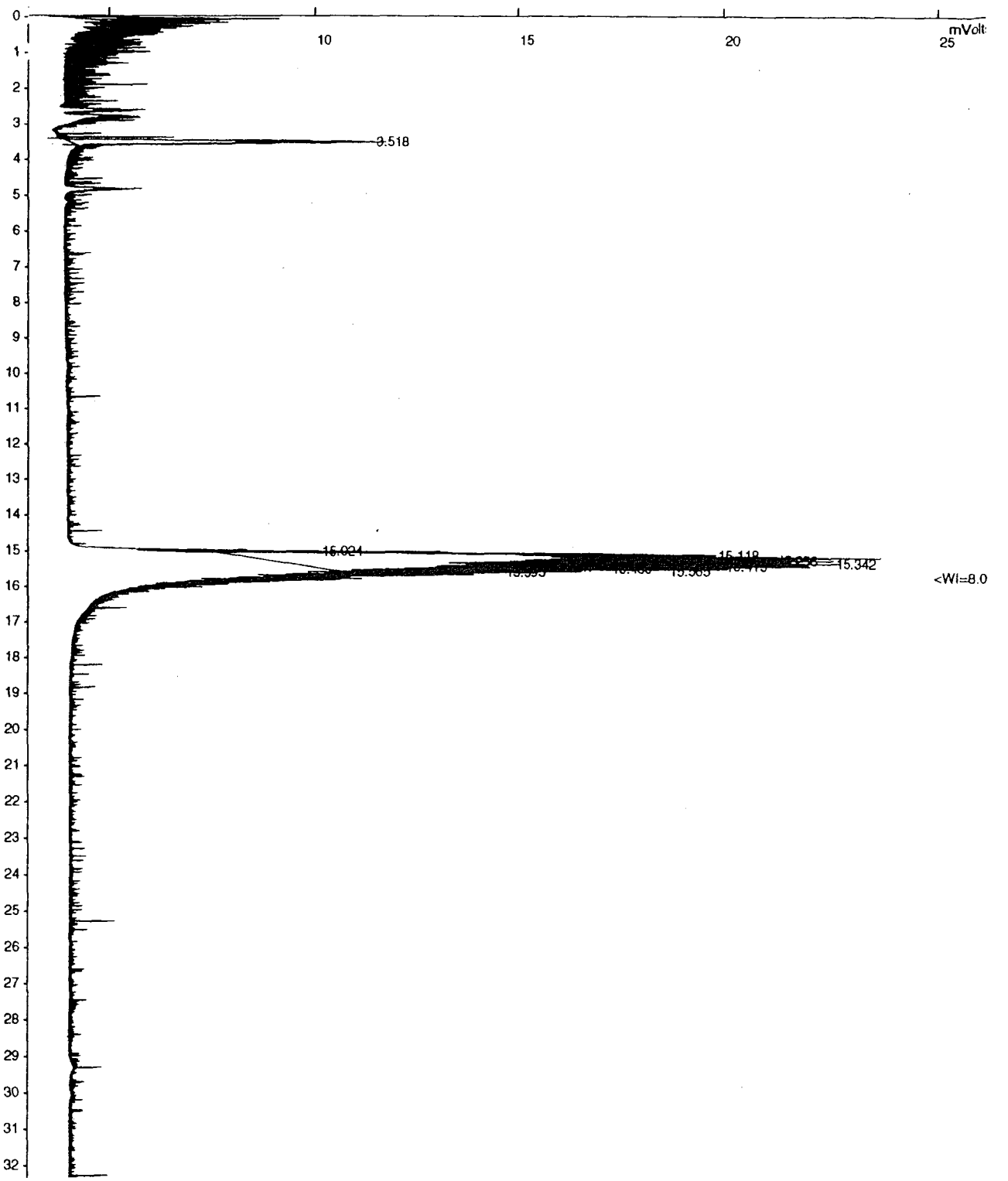
Injection Date: 5/11/04 10:46 AM Calculation Date: 5/11/04 11:20 AM

Operator :
Workstation:
Instrument : Varian Star #1
Channel : 1 = INTGR 1

Detector Type: ProStar/Dynamax (2 Volts)
Bus Address : 24
Sample Rate : 20.00 Hz
Run Time : 32.985 min

** Star Chromatography Workstation Version 5.31 ** 00478-7120-600-00D4 **

Chart Speed = 0.61 cm/min Attenuation = 38 Zero Offset = -13%
Start Time = 0.000 min End Time = 32.980 min Min / Tick = 1.00



Title :
Run File : c:\star\data\hinko\5-11-04,ghrelin time-0.run
Method File : c:\star\30-60%b-30',fr-0.5, 215nm, teap.mth
Sample ID : Ghrelin time-0

Injection Date: 5/11/04 10:46 AM Calculation Date: 5/11/04 11:20 AM

Operator : Detector Type: ProStar/Dynamax (2 Volts)
Workstation: Bus Address : 24
Instrument : Varian Star #1 Sample Rate : 20.00 Hz
Channel : 1 = INTGR 1 Run Time : 32.985 min

** Star Chromatography Workstation Version 5.31 ** 00478-7120-600-00D4 **

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		4.2282	0.053	0.000	12219	BB	0.0	
2		11.8791	3.518	0.000	34328	BB	5.8	
3		0.7957	15.024	0.000	2300	BV	0.0	
4		13.2979	15.118	0.000	38429	VV	6.9	
5		37.5173	15.256	0.000	108418	VV	0.0	
6		7.1107	15.342	0.000	20549	VV	0.0	
7		8.9862	15.398	0.000	25968	VV	0.0	
8		2.3252	15.415	0.000	6719	VV	0.0	
9		4.5049	15.447	0.000	13018	VV	0.0	
10		4.0970	15.489	0.000	11840	VV	0.0	
11		4.1772	15.565	0.000	12071	VV	12.3	
12		1.0807	15.595	0.000	3123	VB	0.0	
	Totals:	100.0001		0.000	288982			

Total Unidentified Counts : 288981 counts

Detected Peaks: 12 Rejected Peaks: 0 Identified Peaks: 0

Multiplier: 1 Divisor: 1 Unidentified Peak Factor: 0

Baseline Offset: 3723 microVolts

Noise (used): 43 microVolts - monitored before this run

Manual injection

****

Run File : c:\star\data\hinko\5-11-04,ghrelin - time 45'.run
Method File : c:\star\30-60%b-30',fr-0.5, 215nm, teap.mth
Sample ID : Ghrelin - time 45'

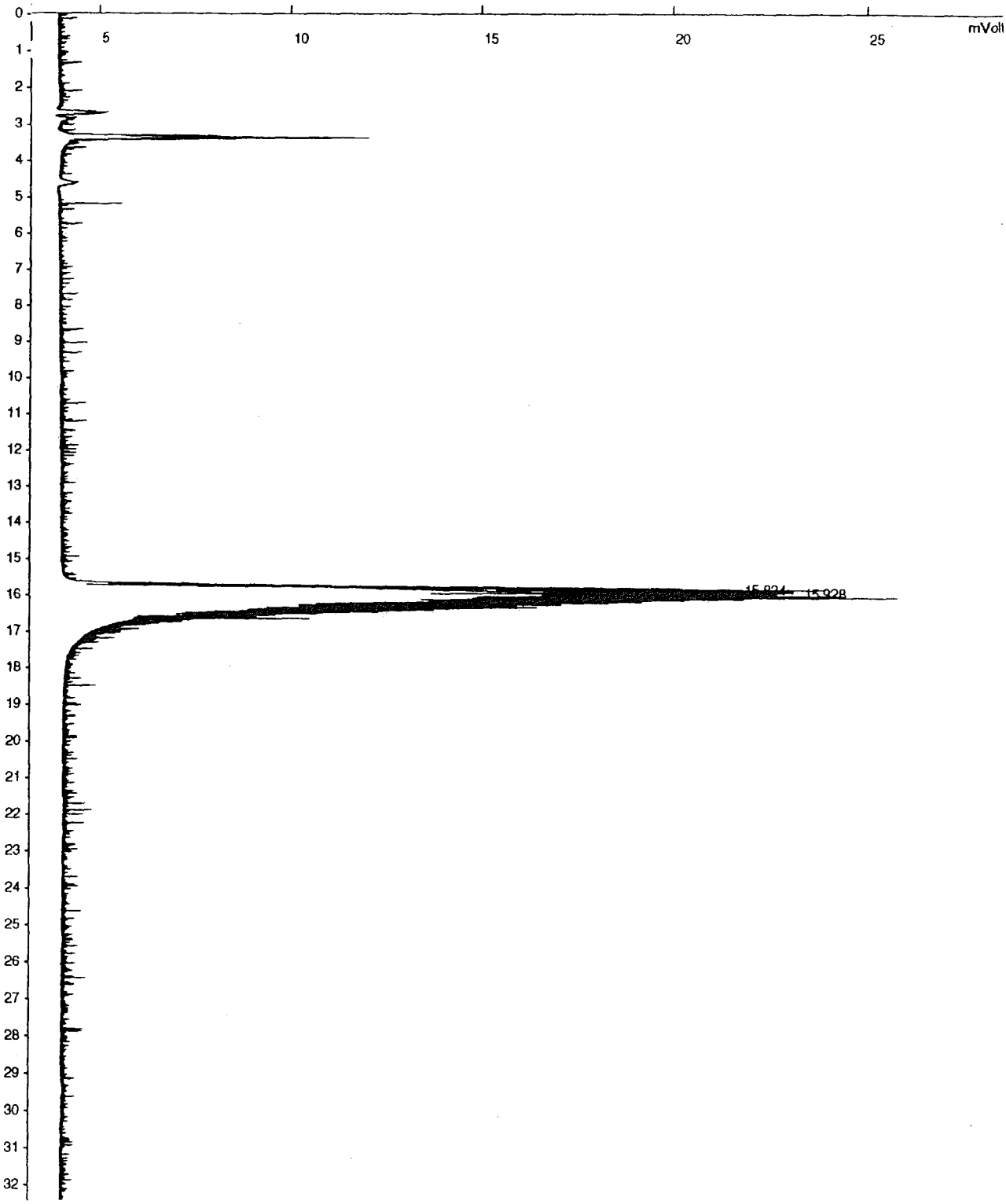
Injection Date: 5/11/04 11:32 AM Calculation Date: 5/11/04 12:06 PM

Operator :
Workstation:
Instrument : Varian Star #1
Channel : 1 = INTGR 1

Detector Type: ProStar/Dynamax (2 Volts)
Bus Address : 24
Sample Rate : 20.00 Hz
Run Time : 32.987 min

** Star Chromatography Workstation Version 5.31 ** 00478-7120-600-00D4 **

Chart Speed = 0.61 cm/min Attenuation = 41 Zero Offset = -12%
Start Time = 0.000 min End Time = 32.980 min Min / Tick = 1.00



Title :
Run File : c:\star\data\hinko\5-11-04,ghrelin - time 45'.run
Method File : c:\star\30-60%b-30',fr-0.5, 215nm, teap.mth
Sample ID : Ghrelin - time 45'

Injection Date: 5/11/04 11:32 AM Calculation Date: 5/11/04 12:06 PM

Operator :
Workstation:
Instrument : Varian Star #1
Channel : 1 = INTGR 1
Detector Type: ProStar/Dynamax (2 Volts)
Bus Address : 24
Sample Rate : 20.00 Hz
Run Time : 32.987 min

** Star Chromatography Workstation Version 5.31 ** 00478-7120-600-00D4 **

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		59.1217	15.834	0.000	26498	BV	5.2	
2		40.8783	15.928	0.000	18321	VB	0.0	
	Totals:	100.0000		0.000	44819			

Total Unidentified Counts : 44819 counts

Detected Peaks: 2 Rejected Peaks: 0 Identified Peaks: 0

Multiplier: 1 Divisor: 1 Unidentified Peak Factor: 0

Baseline Offset: 4088 microVolts

Noise (used): 77 microVolts - monitored before this run

Manual injection

****

Run File : c:\star\data\hinko\5-11-04,ghrelin, time 90min.run
Method File : C:\Star\30-60%B-30',FR-0.5, 215nm, TEAP.mth
Sample ID : Ghrelin, time 90min

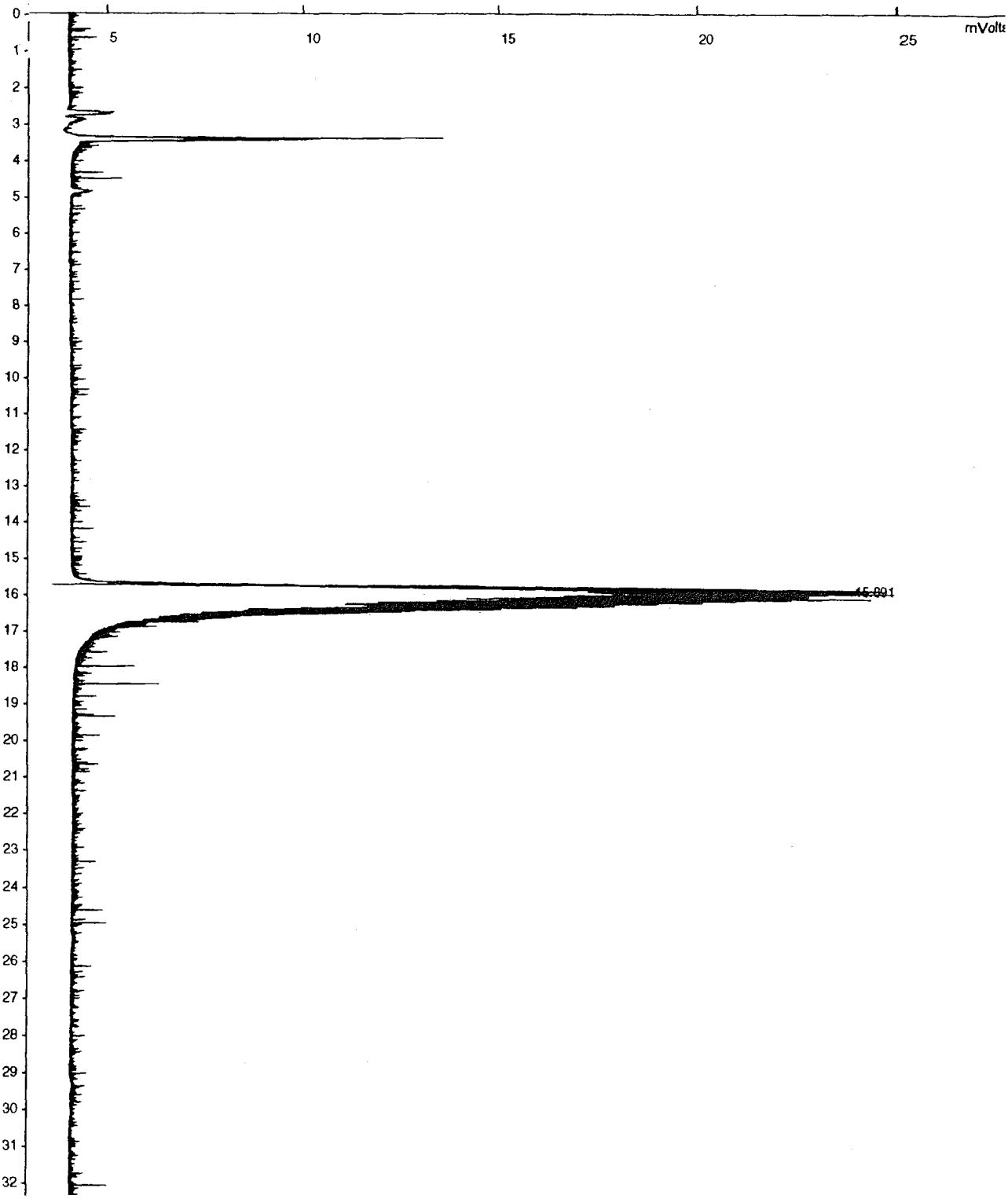
Injection Date: 5/11/04 12:20 PM Calculation Date: 5/11/04 12:53 PM

Operator :
Workstation:
Instrument : Varian Star #1
Channel : 1 = INTGR 1

Detector Type: ProStar/Dynamax (2 Volts)
Bus Address : 24
Sample Rate : 20.00 Hz
Run Time : 32.984 min

** Star Chromatography Workstation Version 5.31 ** 00478-7120-600-00D4 **

Chart Speed = 0.61 cm/min Attenuation = 40 Zero Offset = -12%
Start Time = 0.000 min End Time = 32.980 min Min / Tick = 1.00



Title :
Run File : c:\star\data\hinko\5-11-04,ghrelin, time 90min.run
Method File : C:\Star\30-60%B-30',FR-0.5, 215nm, TEAP.mth
Sample ID : Ghrelin, time 90min

Injection Date: 5/11/04 12:20 PM Calculation Date: 5/11/04 12:53 PM

Operator :
Workstation:
Instrument : Varian Star #1
Channel : 1 = INTGR 1
Detector Type: ProStar/Dynamax (2 Volts)
Bus Address : 24
Sample Rate : 20.00 Hz
Run Time : 32.984 min

** Star Chromatography Workstation Version 5.31 ** 00478-7120-600-00D4 **

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		100.0000	15.891	0.000	42176	BB	0.0	
	Totals:	100.0000		0.000	42176			

Total Unidentified Counts : 42176 counts

Detected Peaks: 1 Rejected Peaks: 0 Identified Peaks: 0

Multiplier: 1 Divisor: 1 Unidentified Peak Factor: 0

Baseline Offset: 4035 microVolts

Noise (used): 77 microVolts - monitored before this run

Manual injection

****

Run File : c:\star\data\hinko\5-11-04,ghrelin, time -135'.run
Method File : C:\Star\30-60%B-30',FR-0.5, 215nm, TEAP.mth
Sample ID : Ghrelin, time -135'

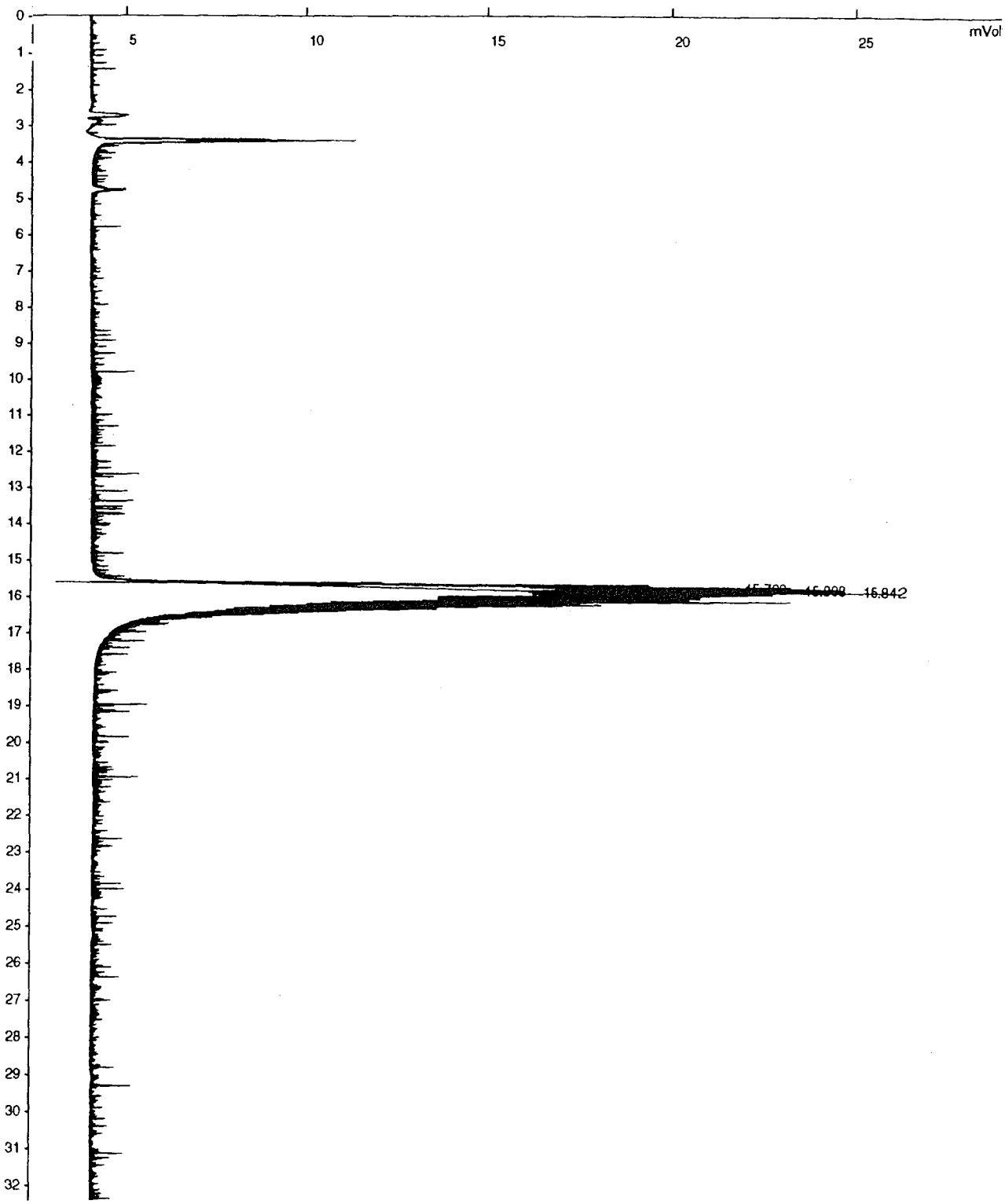
Injection Date: 5/11/04 1:07 PM Calculation Date: 5/11/04 1:40 PM

Operator :
Workstation:
Instrument : Varian Star #1
Channel : 1 = INTGR 1

Detector Type: ProStar/Dynamax (2 Volts)
Bus Address : 24
Sample Rate : 20.00 Hz
Run Time : 32.987 min

** Star Chromatography Workstation Version 5.31 ** 00478-7120-600-00D4 **

Chart Speed = 0.61 cm/min Attenuation = 43 Zero Offset = -9%
Start Time = 0.000 min End Time = 32.980 min Min / Tick = 1.00



Title :
Run File : c:\star\data\hinko\5-11-04,ghrelin, time -135'.run
Method File : C:\Star\30-60%B-30',FR-0.5, 215nm, TEAP.mth
Sample ID : Ghrelin, time -135'

Injection Date: 5/11/04 1:07 PM Calculation Date: 5/11/04 1:40 PM

Operator :
Workstation:
Instrument : Varian Star #1
Channel : 1 = INTGR 1
Detector Type: ProStar/Dynamax (2 Volts)
Bus Address : 24
Sample Rate : 20.00 Hz
Run Time : 32.987 min

** Star Chromatography Workstation Version 5.31 ** 00478-7120-600-00D4 **

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		71.3389	15.763	0.000	60034	BV	10.0	
2		13.7493	15.808	0.000	11571	VV	10.0	
3		14.9118	15.842	0.000	12549	VB	0.0	
	Totals:	100.0000		0.000	84154			

Total Unidentified Counts : 84153 counts

Detected Peaks: 3 Rejected Peaks: 0 Identified Peaks: 0

Multiplier: 1 Divisor: 1 Unidentified Peak Factor: 0

Baseline Offset: 4022 microVolts

Noise (used): 71 microVolts - monitored before this run

Manual injection

****

Run File : c:\star\data\hinko\5-11-04,ghrelin - time 180'.run
Method File : c:\star\30-60%b-30',fr-0.5, 215nm, teap.mth
Sample ID : Ghrelin - time 180'

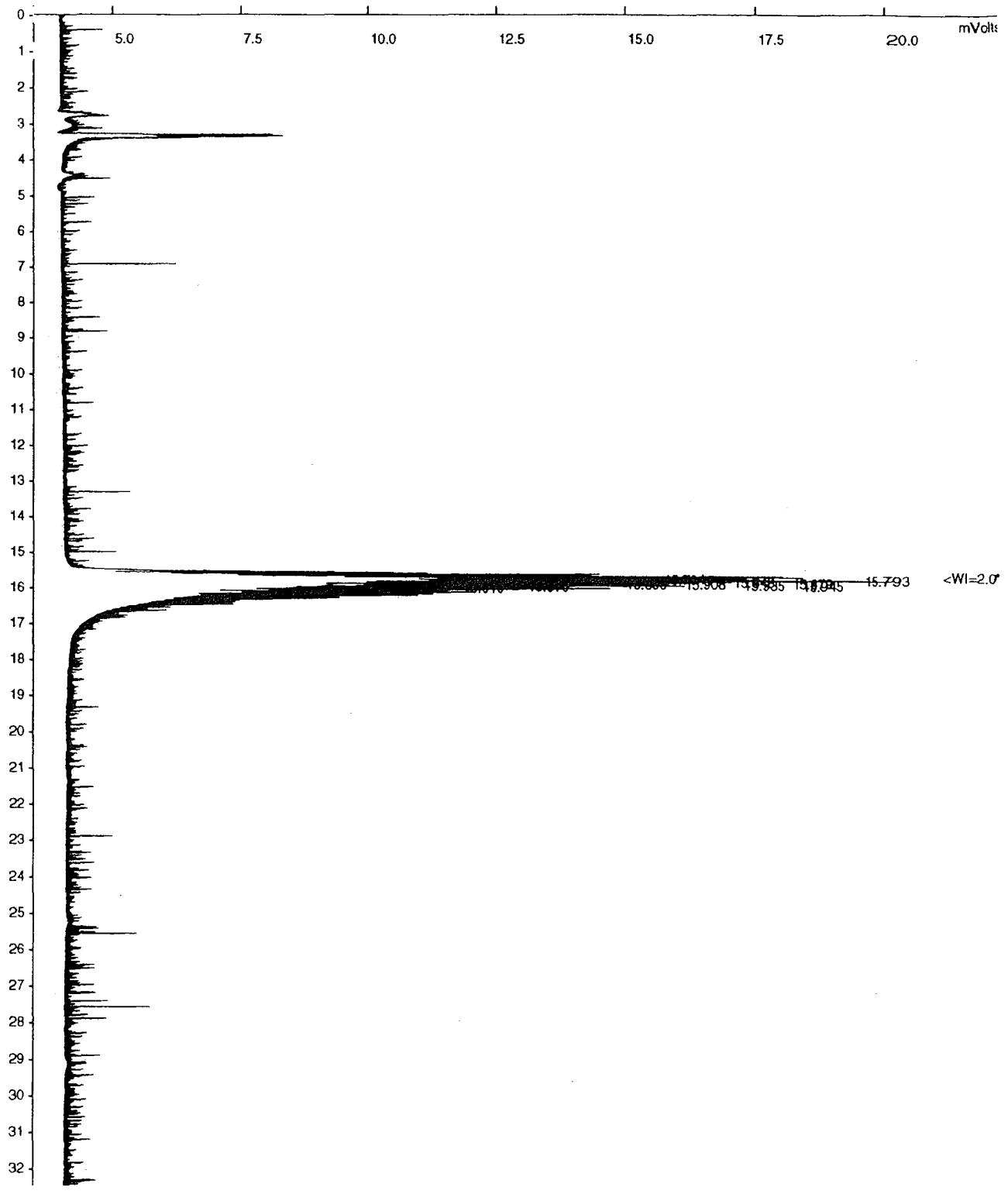
Injection Date: 5/11/04 1:54 PM Calculation Date: 5/11/04 2:34 PM

Operator :
Workstation:
Instrument : Varian Star #1
Channel : 1 = INTGR 1

Detector Type: ProStar/Dynamax (2 Volts)
Bus Address : 24
Sample Rate : 20.00 Hz
Run Time : 32.986 min

** Star Chromatography Workstation Version 5.31 ** 00478-7120-600-00D4 **

Chart Speed = 0.61 cm/min Attenuation = 30 Zero Offset = -18%
Start Time = 0.000 min End Time = 32.980 min Min / Tick = 1.00



Title :
Run File : c:\star\data\hinko\5-11-04,ghrelin - time 180'.run
Method File : c:\star\30-60%b-30',fr-0.5, 215nm, teap.mth
Sample ID : Ghrelin - time 180'

Injection Date: 5/11/04 1:54 PM Calculation Date: 5/11/04 2:34 PM

Operator :
Workstation:
Instrument : Varian Star #1
Channel : 1 = INTGR 1

Detector Type: ProStar/Dynamax (2 Volts)
Bus Address : 24
Sample Rate : 20.00 Hz
Run Time : 32.986 min

** Star Chromatography Workstation Version 5.31 ** 00478-7120-600-00D4 **

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		10.2307	15.764	0.000	3275	BV	0.2	
2		7.9594	15.793	0.000	2548	VB	0.6	
3		6.6585	15.843	0.000	2131	BV	0.5	
4		4.1594	15.879	0.000	1331	BV	0.0	
5		10.7201	15.899	0.000	3431	VV	0.1	
6		6.5792	15.908	0.000	2106	VV	0.4	
7		19.4647	15.935	0.000	6230	VV	0.2	
8		8.6039	15.945	0.000	2754	VV	0.3	
9		14.3596	15.970	0.000	4596	VB	0.0	
10		11.2645	16.010	0.000	3606	BB	0.1	
	Totals:	100.0000		0.000	32008			

Total Unidentified Counts : 32008 counts

Detected Peaks: 14 Rejected Peaks: 4 Identified Peaks: 0

Multiplier: 1 Divisor: 1 Unidentified Peak Factor: 0

Baseline Offset: 4002 microVolts

Noise (used): 76 microVolts - monitored before this run

Manual injection

****

CONSENT FORM
MONTREAL NEUROLOGICAL INSTITUTE AND HOSPITAL
McConnell Brain Imaging Centre

Investigators: Saima Malik, MSc; Alain Dagher, MD; Gloria Tannenbaum, PhD

1. TITLE OF PROJECT

Regional brain activation associated with ghrelin infusion.

2. REASON FOR THE STUDY

Ghrelin is a circulating gastric hormone that has been implicated in feeding behaviour. In humans, ghrelin possesses significant appetite-stimulating effects. We are interested in investigating the link between ghrelin and the brain. Our precise goal is to measure neuronal activation in specific brain regions following ghrelin administration, in healthy adults.

3. PROCEDURES

Your participation in this study will involve 1 fMRI session. During the scan, you will be infused with ghrelin in normal saline, and with normal saline alone. These two conditions will be administered to participants in a random fashion to avoid any possible order effects. Changes in brain activity will be measured using fMRI. Importantly, saline and ghrelin infusions will occur approximately 3 hours after an investigator-provided standard test breakfast (specific caloric intake), subsequent to an overnight fast. Also, three blood samples (7 ml each) will be collected at specific time points (morning of study, 15 min pre-injection and 90-min post-infusion). The total volume of blood drawn will approximately be 21 ml (or 1.5 tablespoons). One additional sample will be drawn for a routine blood analysis. Finally, you will be asked to fill out a few standardised questionnaires examining various socio-demographic variables. The time commitment is approximately 5 ½ hours

In terms of the scanning protocol, you will be asked to lie on a couch that will be moved into a cylindrical opening where pictures of your head will be taken during an estimated period of 50 minutes. The MRI machine will be quite noisy during the scan hence; you will be given earplugs. During the procedure, you will be able to communicate with the technician.

4. CONTRAINDICATIONS

The following are contraindications for a magnetic resonance study:

- Pacemaker
- Aneurysm Clip
- Heart/Vascular Clip
- Prosthetic Valve
- Metal Prosthesis
- Pregnancy
- Claustrophobia
- Metal fragments in body

5. ADVANTAGES OF THE PROPOSED STUDY

MRI studies are tests, not treatments. It is hoped that the information obtained will help our understanding of the function of the human brain. This may, in the long term, help the diagnosis and treatment of neurological disorders.

6. DISADVANTAGES OF THE PROPOSED STUDY

During the MRI scans, you will be exposed to a strong magnetic field. No long-term negative side effects have been observed from such exposure. As mentioned above, the MR is very noisy and you will be given earplugs to reduce this effect. Also, ghrelin infusion has previously been reported to stimulate appetite in healthy humans. Hence, you may experience hunger subsequent to ghrelin administration. Finally, although blood sampling is a routine procedure with minimal risk, certain side effects are possible. In particular, bruising and bleeding around the site, discomfort, fainting and rarely infection.

7. CONFIDENTIAL NATURE OF THIS STUDY

The results of the testing will be kept confidential. No personal information will be released to third parties without your written consent. Please note however that the Research Ethics Board or Quality Assurance Officers duly authorized by it may access study data.

8. DISCONTINUATION OF THE STUDY BY THE INVESTIGATOR

At any time during the testing, the investigators have the right to terminate the study.

9. WITHDRAWAL FROM THE STUDY

Your participation in this research project is voluntary and you may withdraw at any time, including during the procedure, without prejudice to yourself, or your treatment.

10. INCIDENTAL FINDINGS

Research scans are not subject to clinical review. However, any incidental findings will be communicated to you and, upon your request, to your physician.

11. EFFECTS OF PARTICIPATION IN THIS STUDY

Magnetic resonance imaging does not interfere with any treatment or other diagnostic tests.

12. CONTACT BY THE RESEARCH ETHICS BOARD

You may be may be contacted by a member of the Research Ethics Board, at the discretion of the board.

13. COMPENSATION FOR PARTICIPATION IN THE STUDY

Upon completion of both MRI studies you will receive $100, as compensation for your time and inconvenience. If the experiment(s) have

to be terminated, compensation will be adjusted according to the fraction of the studies completed.

14. CONTACT INFORMATION FOR SUBJECT

You may contact Dr. Alain Dagher and/or Saima Malik at the MNI (514-398-1996), concerning, any later inquiries about the study. Furthermore, you may inquire about your rights or lodge eventual complaints with the MNH patient's committee, Room 354, tel. (514) 398-5358.

15. FUNDING SOURCE

This study will be funded by a grant from Unilever PLC (UK) to Dr. Dagher.

Regional Brain Activation Associated with Ghrelin Infusion

DECLARATION OF CONSENT

McConnell Brain Imaging Centre, Montreal Neurological Institute

I, _____, have reviewed the questions on page 3 with one of the investigators, _____.

I fully understand the procedures, advantages and disadvantages of the MRI study which have been explained to me. I freely and voluntarily consent to participate in this study.

Further, I understand that I may seek information about this test either before or after it is given, that I am free to withdraw from the testing at any time if I desire, and that my personal information will be kept confidential.

SIGNATURE _____

SUBJECT *DATE* *CONTACT NO.*

SIGNATURE _____

INVESTIGATOR *DATE* *CONTACT NO.*

Regional Brain Activation Associated with Ghrelin Infusion

Magnetic Resonance Imaging
QUESTIONNAIRE
McConnell Brain Imaging Centre, Montreal Neurological Institute

It is of the **utmost importance** for the subject that this questionnaire, be completed by the **subject and investigator**.

1. Previous surgery (type and date)

_____

_____

2. Does the subject have any of the following?	**YES**	**NO**
Cardiac pacemaker	____	____
Surgical clip on an aneurysm or other vessel	____	____
Surgical clip or valve on the heart	____	____
Prostheses (please specify type and location) _____	____	____
Implants (please specify type and location) _____	____	____
Metal or metallic fragments in any part of the body (please specify) _____	____	____
3. Is the subject pregnant?	____	____

SIGNATURE

_____

SUBJECT *DATE* *CONTACT NO.*

SIGNATURE

_____

INVESTIGATOR *DATE* *CONTACT NO.*

FORMULAIRE DE CONSENTEMENT
INSTITUT ET HÔPITAL NEUROLOGIQUES DE MONTRÉAL
Centre d'imagerie cérébrale McConnell

Chercheurs : Saima Malik, MSc; Alain Dagher, MD; Gloria Tannenbaum, PhD

1. TITRE DU PROJET

Activation des régions du cerveau associées à l'infusion de ghreline.

2. MOTIF DE L'ÉTUDE

La ghreline est une hormone circulant dans l'estomac et elle est impliquée dans le contrôle de l'appétit. Chez les humains, la ghreline possède des effets significatifs de stimulation de l'appétit. Nous sommes intéressés à enquêter sur le lien entre la ghreline et le cerveau. Notre but précis consiste à mesurer l'activation neuronique dans des régions cérébrales précises suite à l'administration de ghreline à des adultes en santé.

3. PROCÉDURES

Votre participation à cette étude inclura une session d'IRMf. Pendant la session, vous recevrez une infusion de ghreline dans une solution saline, et aussi une infusion de solution saline. Ces deux conditions seront administrées d'une façon aléatoire afin d'éviter tout effet possible de classement. Les changements dans l'activité cérébrale seront mesurés en utilisant l'IRMf. Il est important de savoir que les infusions de solution saline ou de ghreline surviendront environ 3 heures après que vous aurez consommé un petit déjeuner pré test standard (contenant nombre spécifique de calories) qui est fourni par le chercheur et ce, après avoir passé la nuit sans manger. De plus, trois échantillons de sang (de 7 ml chacune) seront prélevés à des intervalles spécifiques (soit le matin, 15 minutes avant l'injection, et à 90 minutes après l'injection). La quantité totale de sang obtenue sera approximativement de 21 ml (ou 1.5 cuillerées à table). Un autre échantillon sera prélevé pour un analyse de sang. Finalement, on vous demandera de remplir quelques questionnaires portant sur diverses données socio-démographiques. Le temps requis pour ce projet est d'environ 5 ½ heures.

En terme de protocole d'imagerie par résonance magnétique, on vous demandera de vous allonger sur la table de l'appareil que l'on fera glisser dans une ouverture cylindrique pour prendre des images de votre tête d'une durée approximative de 50 minutes. La machine IRM fait beaucoup de bruit durant cette opération et pour atténuer ce bruit, on vous donnera des bouchons pour les oreilles. Au cours de la session il vous sera possible de communiquer avec le technicien.

4. CONTRE-INDICATIONS

- Stimulateur cardiaque
- Clip d'anévrisme
- Clip cardiaque ou vasculaire
- Valve artificielle
- Prothèses métalliques
- Grossesse
- Claustrophobie
- Fragments métalliques au corps

5. AVANTAGES DE L'ÉTUDE PROPOSÉE

L'imagerie par résonance magnétique est un examen et non un traitement. Nous espérons que les renseignements recueillis nous aideront à mieux comprendre le fonctionnement du cerveau humain. Cela pourrait, à long terme, contribuer au diagnostic et au traitement de troubles neurologiques.

6. INCONVÉNIENTS DE L'ÉTUDE PROPOSÉE

Pendant l'examen d'IRM, vous serez exposé à un champ magnétique puissant. Aucun effet secondaire à long terme n'a été observé à l'issue de ce type d'étude. Tel qu'indiqué ci-dessus, la machine est très bruyante et nous vous donnerons des bouchons pour les oreilles. Il nous est rapporté que l'infusion de ghreline stimule l'appétit chez l'humain en bonne santé. Donc, il est possible que vous ayez faim après l'administration de la ghreline. Bien que la prise de sang soit une procédure routinière à risque minime, elle peut causer certains effets secondaires. Particulièrement, des saignements au point d'entrée de l'aiguille, des ecchymoses, un inconfort, des étourdissements et assez rarement de l'infection.

7. CARACTÈRE CONFIDENTIEL DE L'ÉTUDE

Les résultats de cette étude resteront confidentiels. Aucune données vous concernant ne sera transmise à un tiers sans votre autorisation écrite. Veuillez noter toutefois que le Comité d'éthique de la recherche ou des membres du personnel de l'Assurance de Qualité qui sont autorisés par ce comité peuvent avoir accès aux données de l'étude.

8. INTERUPTION DE L'ÉTUDE PAR LE CHERCHEUR

Le chercheur a le droit de mettre fin à cette étude à tout moment.

9. RETRAIT DE L'ÉTUDE

Votre participation à ce projet de recherche est volontaire et vous pouvez vous en retirer à tout moment, incluant durant la procédure et ceci sans préjudice, ni pour vous-mêmes ni pour vos traitements.

10. CONSTATATIONS FORTUITES

Les résultats d'IRM provenant de protocoles de recherche ne sont pas soumis à une évaluation clinique. Cependant, toute constatation fortuite concernant votre santé sera portée à votre connaissance et, si vous en faites la demande, à celle de votre médecin.

11. EFFET DE VOTRE PARTICIPATION SUR VOTRE TRAITEMENT

L'imagerie par résonance magnétique ne nuit à aucun traitement ou test diagnostique.

12. CONTACT PAR LE COMITÉ D'ÉTHIQUE DE LA RECHERCHE

Je comprends qu'un membre du comité d'éthique peut communiquer avec moi et ce à la discrétion du comité.

13. COMPENSATION POUR PARTICIPER À CE PROJET

A l'achèvement des deux études d'IRM, vous toucherez $100 à titre compensatoire pour votre temps et tout inconvénient. Advenant qu'il faille mettre fin à ce projet, votre compensation sera fonction de la fraction des études terminée.

14. PERSONNE RESSOURCE POUR LE SUJET

Il est entendu que je peux communiquer avec le Dr Alain Dagher ou avec Saima Malik à l'Institut et hôpital neurologiques de Montréal (514-398-1743) pour tous renseignements concernant ce projet. De plus, pour tout renseignements à propos de vos droits, ou encore pour formuler une plainte le cas échéant, veuillez vous adresser au Comité des patients de hôpital neurologiques de Montréal, bureau 354, tél. (514) 398-5358.

15. SUBVENTIONNÉ PAR

Cette étude sera financée par une subvention accordée au Dr Dagher par Unilever PLC (UK).

Activation des Régions du Cerveau Associées à l'Infusion de Ghréline

DÉCLARATION DE CONSENTEMENT

Centre d'Imagerie Cérébrale McConnell, Institut et Hôpital Neurologiques de Montréal

Je soussigné(e) _____ ai révisé les questions de la page 3 en présence des chercheurs suivants _____.

J'ai parfaitement compris les procédures, les avantages et les inconvénients de cette étude de résonance magnétique qui m'ont d'ailleurs été expliqués. Je consens librement et volontairement à y participer.

Il est entendu par ailleurs que je peux demander des renseignements à propos de cet examen avant ou après son déroulement, que je suis libre de me retirer de ce protocole à tout moment si je le souhaite et que toutes données me concernant resteront confidentielles.

SIGNATURE

_____

SUJET *DATE* *N^{O} DE CONTACT*

SIGNATURE

_____

CHERCHEUR *DATE* *N^{O} DE CONTACT*

Activation des Régions du Cerveau Associées à l'Infusion de Ghréline

Imagerie par résonance magnétique (IRM)
QUESTIONNAIRE
Centre d'imagerie cérébrale McConnell, Institut et hôpital neurologiques de Montréal

Il est **essentiel** pour le sujet que ce questionnaire **soit rempli** par le **sujet ainsi** que par le **chercheur**.

1. Chirurgies antérieures (type et date)

_____

_____

2. Le sujet porte-t-il l'un ou plusieurs des éléments suivants?

	OUI	**NON**
Stimulateur cardiaque	_____	_____
Clip d'anévrisme ou clip sur un autre vaisseau	_____	_____
Clip chirurgical ou valve cardiaque	_____	_____
Prothèse (veuillez préciser le type et l'organe) _____	_____	_____
Implants (veuillez préciser le type et l'organe) _____	_____	_____
Métal ou fragments métalliques dans le corps (veuillez préciser) _____	_____	_____

3. Le sujet est-elle enceinte? _____ _____

SIGNATURE

_____

SUJET *DATE* *CONTACT NO.*

SIGNATURE

_____

CHERCHEUR *DATE* *CONTACT NO.*

Study 2

Saima Malik, Miss

From: Alain Dagher [alain.dagher@mcgill.ca] **Sent:** Wed 28/05/2008 7:00 PM
To: Saima Malik, Miss
Cc:
Subject: Re: Waiver for Thesis
Attachments:

On 5/15/08 11:12 AM, "Saima Malik, Miss" <saima.malik@mail.mcgill.ca> wrote:

> Dear co-authors,
>
> I have presently completed the second manuscript, entitled:
>
> **State of Expectancy Modulates the Neural Response to Visual Food Stimuli in Humans**
> **Malik S, McGlone F and Dagher A.**
> This paper will be included in my doctoral thesis. In compliance with McGill Faculty of Graduate Studies and Research guidelines, a *"candidate must ... include signed waivers from any co-authors of unpublished manuscripts"*. I would appreciate it, if you could provide such a waiver by responding to this e-mail.
>
> Many thanks.
>
> Regards,
>
> Saima

I agree to have this paper included in your thesis.

Alain Dagher, MD
Associate Professor
Montreal Neurological Institute
McGill University

3801 University St
Montreal QC
Canada H3A 2B4
tel: (514) 398-1726
fax: (514) 398-8948
alain@bic.mni.mcgill.ca

Saima Malik, Miss

From: Mcglone, Francis [Francis.McGlone@Unilever.com] **Sent:** Sat 17/05/2008 11:35 AM
To: Saima Malik, Miss
Cc:
Subject: RE: Waiver for Thesis
Attachments:

As requested Malik.
Francis

Dr Francis McGlone, Tel: + 44 (0) 151 641 3326
Cognitive Neuroscience mobile: +44 (0) 7711152895
Unilever R&D
Wirral, Cheshire,
CH63 3JW, UK

Visiting Professor: Dept. Neurological Sciences, School of Medicine, Liverpool University, UK
Special Professor: Sir Peter Mansfield Magnetic Resonance Centre, University of Nottingham, UK

Nicky Roberts Tel: + 44 (0) 151 641 1251
Secretary. email: nicky.roberts@unilever.com

-----Original Message-----
From: Saima Malik, Miss [mailto:saima.malik@mail.mcgill.ca]
Sent: Thursday, May 15, 2008 4:12 PM
To: Alain Dagher, Dr.; francis.mcglone@liverpool.ac.uk; Mcglone, Francis
Subject: Waiver for Thesis

Dear co-authors,

I have presently completed the second manuscript, entitled:

State of Expectancy Modulates the Neural Response to Visual Food Stimuli in Humans

Malik S, McGlone F and Dagher A.

This paper will be included in my doctoral thesis. In compliance with McGill Faculty of Graduate Studies and Research guidelines, a "candidate must ... include signed waivers from any co-authors of unpublished manuscripts". I would appreciate it, if you could provide such a waiver by responding to this e-mail.

Many thanks.

Regards,

Saima

--
Saima Malik, MSc
McGill University
Montreal Neurological Institute

3801 University Street
Montreal, Quebec, H3A-2B4
Tel. (514) 398-1743
Fax. (514) 398-8948

MAGNETIC RESONANCE IMAGING (MRI)
CONSENT FORM
MONTREAL NEUROLOGICAL INSTITUTE AND HOSPITAL
McConnell Brain Imaging Centre

Investigators: Saima Malik, MSc; Alain Dagher, MD; Gloria Tannenbaum, PhD

1. TITLE OF PROJECT

Regional brain activation associated with food craving.

2. REASON FOR THE STUDY

Food craving is defined as an intense desire to eat a certain food or type of food. The aim of this study is to test the hypothesis that cue-elicited food craving activates the mesolimbic dopamine system. The precise goal is to measure neuronal activation in specific brain regions in response to pictures of food and neutral objects.

3. PROCEDURES

Your participation in this study will involve two imaging sessions, each subsequent to an 8-hour fast. During the scans, you will be exposed to pictures of food and neutral images. Changes in brain activity will be measured using functional Magnetic Resonance Imaging (MRI). While in the scanner, you will also be asked to respond to approximately 10 questions regarding your mood and appetite. Responses will be recorded via a computer mouse. In addition, three blood samples (7 ml each) will be collected at specific time points, during each session. The total volume of blood drawn will approximately be 21 ml (or 1.5 tablespoons) per session. Finally, you will be asked to fill out a few standardised questionnaires examining various socio-demographic variables. The time commitment for this study is approximately 4 hours.

In terms of the scanning protocol, you will be asked to lie on a couch that will be moved into a cylindrical opening where pictures of your head will be taken. The MRI machine will be quite noisy during the scan hence, you will be given earplugs. During the procedure, you will be able to communicate with the technician.

4. CONTRAINDICATIONS

The following are contraindications for a magnetic resonance study:

- Pacemaker
- Aneurysm Clip
- Heart/Vascular Clip
- Prosthetic Valve
- Metal Prosthesis
- Pregnancy
- Claustrophobia
- Metal fragments in body

5. ADVANTAGES OF THE PROPOSED STUDY

MRI studies are tests, not treatments. It is hoped that the information obtained will help our understanding of the function of the human brain.

This may, in the long term, help the diagnosis and treatment of neurological disorders.

6. DISADVANTAGES OF THE PROPOSED STUDY

During the MRI scans, you will be exposed to a strong magnetic field. No long-term negative side effects have been observed from such exposure. As mentioned above, the MR is very noisy and you will be given earplugs to reduce this effect. Also, although blood sampling is a routine procedure with minimal risk, certain side effects are possible. In particular, bruising and bleeding around the site, discomfort, fainting and rarely infection.

7. CONFIDENTIAL NATURE OF THIS STUDY

The results of the testing will be kept confidential. No personal information will be released to third parties without your written consent.

8. DISCONTINUATION OF THE STUDY BY THE INVESTIGATOR

At any time during the testing, the investigators have the right to terminate the study.

9. SUBJECT'S STATEMENT CONCERNING WITHDRAWAL FROM THE STUDY

I understand that my participation in this research project is voluntary and I may withdraw at any time, including during the procedure, without prejudice to myself, or my treatment.

10. INCIDENTAL FINDINGS

Research scans are not subject to clinical review. However, any incidental findings will be communicated to you and, upon your request, to your physician.

11. EFFECTS OF PARTICIPATION IN THIS STUDY

Magnetic resonance imaging does not interfere with any treatment or other diagnostic tests

12. SUBJECT'S AGREEMENT TO BE CONTACTED BY THE RESEARCH ETHICS BOARD

I understand that I may be contacted by a member of the Research Ethics Board, at the discretion of the board.

13. COMPENSATION FOR PARTICIPATION IN THE STUDY

Upon completion of both MRI studies you will receive $50, as compensation for your time and inconvenience. If the experiment(s) have to be terminated, compensation will be adjusted according to the fraction of the studies completed.

14. CONTACT INFORMATION FOR SUBJECT

I understand that I may contact Dr. Alain Dagher and/or Saima Malik at the MNI (514-398-1996), concerning, any later inquiries about the study.

15. FUNDING SOURCE

This study will be funded by a grant from Unilever PLC (UK) to Dr. Dagher.

Regional Brain Activation Associated With Food Craving

Magnetic Resonance Imaging
QUESTIONNAIRE

McConnell Brain Imaging Center, Montreal Neurological Institute and Hospital

It is of the **utmost importance** for the subject that this questionnaire, be completed by the **subject and investigator**.

1. Previous surgery (type and date)

_____

_____

2. Does the subject have any of the following?	**YES**	**NO**
Cardiac pacemaker	____	____
Surgical clip on an aneurysm or other vessel	____	____
Surgical clip or valve on the heart	____	____
Prostheses (please specify type and location) _____	____	____
Implants (please specify type and location) _____	____	____
Metal or metallic fragments in any part of the body (please specify) _____	____	____
3. Is the subject pregnant?	____	____

SIGNATURE

_____

SUBJECT *DATE* *CONTACT NO.*

SIGNATURE

_____

INVESTIGATOR *DATE* *CONTACT NO.*

Regional Brain Activation Associated With Food Craving

DECLARATION OF CONSENT

McConnell Brain Imaging Center, Montreal Neurological Institute and Hospital

I, _____, have reviewed the consent documents with one of the investigators, _____.

I fully understand the procedures, advantages and disadvantages of the MRI study which have been explained to me. I freely and voluntarily consent to participate in this study.

Further, I understand that I may seek information about this test either before or after it is given, that I am free to withdraw from the testing at any time if I desire, and that my personal information will be kept confidential.

SIGNATURE _____

SUBJECT *DATE* *CONTACT NO.*

SIGNATURE _____

INVESTIGATOR *DATE* *CONTACT NO.*

IMAGERIE PAR RÉSONANCE MAGNÉTIQUE (IRM)
FORMULAIRE DE CONSENTEMENT
INSTITUT ET HÔPITAL NEUROLOGIQUES DE MONTRÉAL
Centre d'Imagerie Cérébrale McConnell

1. TITRE DU PROJET

Activation des régions du cerveau associées aux envies alimentaires.

2. MOTIF DE L'ÉTUDE

Les envies alimentaires sont un désir irrésistible et impérieux de manger un certain aliment ou un certain type d'aliments. Le but de la présente étude est de tester l'hypothèse que les envies alimentaires engendrées par un indice visuel associé aux aliments désirés, provoque la neurotransmission de la dopamine au sein du système méso limbique. Le but précis est de mesurer l'activation neuronale dans des régions spécifiques du cerveau en réponse à des illustrations d'aliments et d'objets neutres.

3. PROCÉDURES

Votre participation à cette étude comprendra deux sessions d'imagerie médicale précédées d'un jeûne de 8 heures. Pendant les scans, on vous présentera des illustrations d'aliments et des images neutres et les changements d'activité cérébrale seront mesurés par Imagerie par Résonance Magnétique fonctionnelle (IRM). Pendant que vous serez dans le scanner, on vous demandera également de répondre à une dizaine de questions portant sur votre appétit et votre humeur. Vos réponses seront enregistrées à l'aide d'une souris reliée à un ordinateur. Trois échantillons de 7 ml de sang chacun seront également prélevés à des moments spécifiques au cours de chaque session. La quantité totale de sang prélevée sera d'environ 21 ml (soit 1.5 cuillère à table) par session. Finalement, on vous demandera de remplir quelques questionnaires de routine portant sur diverses variantes sociodémographiques. L'étude au complet prendra environ 4 heures de temps.

Pour le protocole du scanner, on vous demandera de vous allonger sur la table de l'appareil que l'on fera glisser dans une ouverture cylindrique pour prendre des images de votre tête. La machine IRM fait beaucoup de bruit durant cette opération et on vous donnera des bouchons pour les oreilles pour atténuer ce bruit. Vous pourrez communiquer avec le technicien tout au long de la procédure.

4. CONTRE-INDICATIONS

- Stimulateur cardiaque
- Clip d'anévrisme
- Clip cardiaque ou vasculaire
- Valve artificielle
- Prothèses métalliques
- Grossesse
- Claustrophobie
- Fragments métalliques dans le corps

5. AVANTAGES DES ÉTUDES PROPOSÉES

L'Imagerie par Résonance Magnétique est un examen et non pas un traitement. Nous espérons que les renseignements obtenus nous aideront à mieux comprendre le fonctionnement du cerveau humain. Cela pourrait à long terme contribuer au diagnostic et au traitement de certains troubles neurologiques.

6. INCONVÉNIENTS DES ÉTUDES PROPOSÉES

Pendant l'examen IRM, vous serez exposé(e) à un champ magnétique puissant. Aucun effet secondaire négatif à long terme n'a été observé à l'issue de ce type d'étude. Comme nous vous l'avons indiqué ci-dessus, la machine est très bruyante et on vous donnera des bouchons pour vos oreilles pour atténuer le bruit.

De plus, bien que les prises de sang soient des procédés de routine ne comportant que des risques minimes, il se peut que vous ressentiez certains effets secondaires tels qu'un hématome ou un saignement à l'endroit de l'insertion, une sensation d'inconfort, un évanouissement et rarement une infection.

7. CARACTÈRE CONFIDENTIEL DE L'ÉTUDE

Les résultats de cette étude resteront confidentiels. Aucune donnée vous concernant ne sera transmise à un tiers sans votre autorisation écrite.

8. INTERRUPTION DE L'ÉTUDE PAR LE CHERCHEUR

Le chercheur a le droit de mettre fin à cette étude à tout moment.

9. DÉCLARATION DES SUJETS QUI SOUHAITENT SE DÉSISTER

Il est entendu que ma participation à ce projet de recherche est purement volontaire et que je peux me désister à tout moment, y compris durant son déroulement, sans que cela ne soit préjudiciable à moi-même ou à mon traitement.

10. CONSTATATIONS FORTUITES

Les scans effectués pour la recherche ne sont pas systématiquement revus par un clinicien. Cependant, toute constatation fortuite sur votre santé sera portée à votre connaissance ou à celle de votre médecin, si vous en faites la demande.

11. EFFET DE VOTRE PARTICIPATION SUR VOTRE TRAITEMENT

L'Imagerie par Résonance Magnétique ne nuit à aucun autre traitement ou test diagnostique.

12. COMITÉ D'ÉTHIQUE

Je comprends qu'il se pourrait qu'un membre du comité d'éthique prenne contact avec moi, si le comité l'estime nécessaire.

13. COMPENSATION POUR VOTRE PARTICIPATION À L'ÉTUDE

A l'achèvement des études IRM, vous toucherez $50 à titre de dédommagement pour votre temps et déplacement. Advenant qu'il faille mettre fin à ces études, votre compensation sera fonction de la fraction des études terminée.

14. PERSONNES RESSOURCES

Il est entendu par ailleurs que si j'ai besoin d'informations additionnelles à propos de cette étude, je peux demander Dr. Alain Dagher ou Saima Malik à l'Institut et Hôpital neurologiques de Montréal (514-398-1996).

15. SOURCE DE FINANCEMENT

Cette étude sera financée par une subvention provenant de Unilever PLC (UK) qui sera versée à Dr Dagher.

Activation des Régions du Cerveau Associées aux Envies Alimentaires

Imagerie par Résonance Magnétique

QUESTIONNAIRE

Centre d'Imagerie Cérébrale McConnell, Institut et Hôpital Neurologiques de Montréal

Il est **essentiel** pour le sujet que ce questionnaire **soit rempli** par le **sujet ainsi** que par le **chercheur**.

1. Chirurgies antérieures (type et date)

_____

_____

2. Le sujet porte-t-il l'un ou plusieurs des éléments suivants?

	OUI	**NON**
Stimulateur cardiaque	_____	_____
Clip d'anévrisme ou clip sur un autre vaisseau	_____	_____
Clip chirurgical ou valve cardiaque	_____	_____
Prothèse (veuillez préciser le type et l'organe) _____	_____	_____
Implants (veuillez préciser le type et l'organe) _____	_____	_____
Métal ou fragments métalliques dans le corps (veuillez préciser) _____	_____	_____

3. Le sujet est-elle enceinte? _____ _____

SIGNATURE

_____

SUJET *DATE* *CONTACT NO.*

SIGNATURE

_____

CHERCHEUR *DATE* *CONTACT NO.*

Activation des Régions du Cerveau Associées aux Envies Alimentaires

DÉCLARATION DE CONSENTEMENT

Centre d'Imagerie Cérébrale McConnell, Institut et Hôpital Neurologiques de Montréal

Je soussigné(e) _____ai pris connaissance des documents de consentement en présente du chercheur suivant:_____.

Je comprends très bien les procédures, les avantages et les inconvénients de cette étude IRM, lesquels m'ont été expliqués. Je consens volontairement et librement à y participer.

Il est par ailleurs entendu que je peux demander des renseignements à propos de ces tests, soit avant ou après leur déroulement, que je suis libre de me désister de ce protocole à tout moment si je le souhaite et que toute donnée me concernant restera confidentielle.

SIGNATURE _____

SUBJET *DATE* *CONTACT NO.*

SIGNATURE _____

CHERCHEUR *DATE* *CONTACT NO.*

DEDICATION

This thesis is dedicated to my parents, Rehana and Salahuddin Malik.