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The Impact of GLP-1 Analog, Exenatide, on The Resting Brain of Lean vs Obese Women

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The Impact of GLP-1 Analog, Exenatide, on The Resting Brain of Lean vs Obese Women

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Molecular, Cellular and Integrative Physiology

By

Kristen Coveleskie

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ABSTRACT OF THE DISSERTATION

The Impact of GLP-1 Analog, Exenatide, on The Resting Brain of Lean vs Obese Women

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Kristen Coveleskie

Doctor of Philosophy in Molecular, Cellular and Integrative Physiology

University of California, Los Angeles, 2016

Professor Emeran Mayer, Chair

Obesity is a growing problem both in The United States and world-wide and women in The United States have the highest mean body mass index (BMI) of high-income countries. Brain imaging studies have provided great insights in the interplay between the gut and the brain in regulation of human ingestive behavior, allowing for the exploration of complex signaling in the brain related to appetite-stimulating cues. Obesity has been viewed as a disruption of the balance between homeostatic processing for energy needs and hedonic processing involved in the rewarding value of food, but these brain differences have yet to be fully examined when it comes to the brain's resting state.

The primary aims of this dissertation were to explore the differences in homeostatic and hedonic brain networks in obese women using resting state techniques to compare frequency oscillations and functional connectivity with lean women at a baseline state and then to determine if functional connectivity would be altered by a variant of a known satiety hormone, GLP-1. In additional, to compare these brain abnormalities with behavioral measures related to appetite. This dissertation also includes a preliminary study examining the impact of high and low calorie beverages on hedonic and homeostatic networks of lean vs obese women while viewing pictures of food which pointed to key abnormalities of the obese woman's brain and an

obese-only discrepancy between brain activations and behavioral measures related to subjective feelings of fullness and appetite.

Our analyses at baseline indicated differences in hedonic regions for overweight and obese women, centering around a key region of reward, the nucleus accumbens which was shown to have an increase in grey matter volume as well as altered frequency distributions in the higher BMI group. Functional connectivity of the nucleus accumbens with other regions in the hedonic network was observed to be greater in the more obese group, a brain pattern also observed in many forms of addiction. Upon injection of the GLP-1 analog, Exenatide, functional connectivity was observed to increase more so in the obese group between key homeostatic regions centered around the Nucleus Tractus Solitaries (NTS). This drug-induced increase in functional connectivity was correlated with an increase of hunger in all subjects but more so in the obese. These results support the notion that Exenatide has an impact on brain connectivity, particularly in the obese and suggest the drug's influence on appetite control might be linked to modified connectivity of an NTS-based network.

This dissertation of Kristen Coveleskie is approved.

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TABLE OF CONTENTS

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CHAPTER ONE

Background: Obesity and the Brain

The Obesity Problem

In many parts of the industrialized world, obesity has reached epidemic proportions. In the United States, thirty four percent of the populations is obese, and an additional 34% is considered overweight(1). Women in the United States have the highest mean Body Mass Index (BMI) of any high income country(2). Obesity-attributable medical expenditures related to the metabolic syndrome and other associated diseases reached $75 billion in the US in 2003(3). Even with the billion-dollar diet industry, long-term weight loss results with dieting have been disappointing. Despite extensive research into possible targets for drug development, only surgical interventions, including gastric bypass surgeries, have shown persistent benefits(4). Due to the high personal and financial burden resulting from obesity, the study of its causes, physiological mechanisms, and potential treatments has become an increasingly important goal of the research community.

Obesity and The Brain: Homeostatic vs Hedonic Drives

Overview

Brain imaging studies in human subjects have made it possible to study the interplay between the gut and the brain in regulation of human ingestive behavior. These techniques have allowed us to elaborate beyond self-report and behavioral responses to look at structural and functional aspects of brain activity with increasing specificity. Ingestive behavior is the integrated response to interoceptive signals, emotion, cognition and reward processes. Alterations in the gut brain interactions and resulting changes in ingestive behavior have been observed in the context of anxiety and depression, as well as in clinical disorders such as functional gastrointestinal and eating disorders(5, 6).

Food intake is subject to intense regulation by homeostatic and hedonic systems. Homeostatic feeding is driven by nutritional or caloric deficiency and involves neuronal signaling from the GI tract to the nucleus of the solitary tract (NTS), hypothalamus, thalamus and posterior insula, known as the primary interoceptive cortex(5, 7). In contrast, hedonic feeding is driven primarily by engagement of central reward circuitry including the nucleus accumbens, amygdala, and orbitofrontal, prefrontal, anterior cingulate, and anterior insular cortices(5, 7). Distinct, but overlapping brain networks are thought to regulate the interplay between these two mechanisms.

The Homeostatic Network and the NTS

The nucleus tractus solitaries (NTS) is a region of the dorsal brainstem that is involved in autonomic processes related to the regulation of cardiovascular, respiratory, and gastrointestinal activities(8). As a primary target of vagal afferents from the gut, the NTS is said to be a key region in regulating homeostatic processing related to hunger and satiety (9, 10). The NTS alone can be responsible for short term satiety-related behaviors (11), and disruption of the connection between the gut and the NTS results in a marked change in eating behavior(12).

The NTS is also a relay station between the body and the rest of the brain, communicating with regions involved in higher level processing in relation to emotional regulation or sensations of hunger. Bi-directional neuronal projections between the NTS and the hypothalamus have been well studied in terms of their influence on sympathetic activities, and the connection between these two regions is thought to play a role in integrating peripheral signals from hormones in regulation of satiety (13). The lateral hypothalamus, in particular, is known as a center for metabolic regulation, with two different sets of neurons either stimulating or restricting eating

behaviors(14). The hypothalamus is a brain region involved in learning, memory, emotion, motivation, and motor responses that all play a role in long-term energy homeostasis(14).

The NTS also communicates with the thalamus. In particular, the paraventricular region of the thalamus receives glutamatergic signals from the NTS that motivate food-seeking behaviors (15, 16). Also, both the NTS and thalamus play a role communicating behavior related to taste (7). The thalamus is another relay station where neuronal input goes on to interact with sensory and interoceptive regions like the insula to create a conscious perception of hunger and taste and feelings of fullness (12). All of these interactions can contribute to both body homeostasis and regulation of eating behaviors.

Hedonic Network and The Nucleus Accumbens

The nucleus accumbens is a region of the ventral striatum that is often referred to as the center for reward or craving in the brain, as it plays a crucial role in coordinating responses to rewarding stimuli. Dopaminergic neurons from the ventral tegmental area (VTA) project to the nucleus accumbens, which stimulates the release of dopamine in response to rewarding stimuli (17). The nucleus accumbens and its influence on dopamine release have been very well studied in relation to reward associated with drug addiction, but the parallels between drug intake and food intake, while suggested, have not been as clearly defined (7, 18).

Region-specific studies have suggested that the nucleus accumbens is involved in processes related to motivation associated with food intake(19) and there is a theory that individual differences in the response of the nucleus accumbens to food cues may be one of the main reasons for obese individuals to show addictive-like behavior, and consequently gain weight (20).

The nucleus accumbens is not solely responsible for the rewarding response to food. The region is involved in a complicated series of bi-directional glutamatergic, dopaminergic and GABAergic innervations between regions such as the amygdala, hippocampus, prefrontal cortices, and anterior cingulate cortex, which work together to determine hedonic response (17). The amygdala and hippocampus are involved with emotional processing and memory and have been linked to food craving (21), the insula is involved in self-reflection and perception, and activation of this region has been associated with the viewing of food images(22), prefrontal cortices are involved in executive control functions, and the anterior cingulate cortex has been linked to compulsivity and lack of control in food intake (23). This network of regions interacts together in the brain to determine and motivate a rewarding response to food.

Dysregulation of Homeostatic and Hedonic Networks in Obesity

While anyone can appreciate varying responses to food based on energy or reward-based needs, studies have suggested a dysregulation of homeostatic and hedonic networks associated with obesity. Structurally, BMI correlates positively with grey matter volume (GMV) in the medial orbitofrontal cortex, hypothalamus, and left putamen(24). Several studies have also identified increases in GMV of the nucleus accumbens in obese subjects in comparison to lean subjects, as well as in subjects who have increased eating behaviors, but similar BMI compared to control subjects(24, 25). It has been suggested that obesity-related structural differences may be a consequence of increased signaling from adipose tissue to the brain(24).

Generally, functional studies have suggested that the obese population relies less on the homeostatic system to regulate food intake. Obese people paradoxically have a decreased level of ghrelin, which is responsible for initiating eating behavior, and normal levels of other peptides (such as leptin) responsible for terminating eating behavior(26). These findings have led some

authors to propose that obese subjects are hyposensitive to, or resistant to homeostatic/interoceptive signals.

In another study in which participants consumed all of their food as a liquid from a concealed dispenser, it was found that lean subjects adjusted their intake to changes in energy density (responding normally to a homeostatic signal), while obese subjects failed to regulate their energy intake through interoceptive mechanisms, again suggesting deficits in homeostatic regulatory mechanisms(27). Thus, the homeostatic network may be more important in regulating eating behavior in lean subjects. Obese subjects might override these physiological signals of hunger and satiety by the greater engagement of the central reward system(28). These findings provide evidence that obese subjects have increased engagement in the hedonic network compared to lean controls. For instance, hunger increases the response of midbrain, amygdala, and ventral striatum to the sight of food(29). Activation of this network is increased in obese compared to lean women(30, 31).

The overarching thesis might be that in lean individuals, homeostatic and hedonic systems interact in a self-regulatory manner in order to produce optimal behavior that results in adequate food intake to keep body weight stable. However, in obese subjects it has been suggested that hedonic mechanisms may override homeostatic control of food intake, resulting in food intake beyond energy requirements(5). Much more work needs to be done to tease out the details, but the concept of obesity as an imbalance of homeostatic and hedonic brain networks seems a good framework to reference.

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CHAPTER TWO

Manuscript: Differences in Brain Responses Between Lean and Obese Women to a Sweetened Drink (published Neurogastroenterol Motil 2013)

Abstract

Background: Ingestion of sweet food is driven by central reward circuits and restrained by endocrine and neurocrine satiety signals. The specific influence of sucrose intake on central affective and reward circuitry and alterations of these mechanisms in the obese are incompletely understood. For this we hypothesized 1) Similar brain regions are engaged by the stimulation of sweet taste receptors by sucrose and by non-nutrient sweeteners and 2) During visual food related cues, obese subjects show greater brain responses to sucrose compared to lean controls. **Methods:** In a double blind, crossover design, 10 obese and 10 lean healthy females received a sucrose or a non-nutrient sweetened beverage prior to viewing food or neutral images. BOLD signal was measured using a 1.5 Tesla MRI scanner. **Key Results:** Viewing food images after ingestion of either drink was associated with engagement of similar brain regions (amygdala, hippocampus, thalamus, anterior insula). Obese differed from lean subjects in behavioral and brain responses rating both beverages as less tasteful and satisfying, yet demonstrating greater brain responses. Obese subjects also showed engagement of an additional brain network (including anterior insula, anterior cingulate, hippocampus, and amygdala) only after sucrose ingestion. **Conclusions & Inferences:** Obese subjects had a reduced behavioral hedonic response yet a greater engagement of affective brain networks, particularly after sucrose ingestion, suggesting in obese subjects, lingual and gut derived signaling generate less central hedonic effects than food-related memories in response to visual cues, analogous to response patterns implicated in food addiction.

Introduction

In many parts of the industrialized world, obesity has reached epidemic proportions. In the United States, thirty four percent of the population is obese and an additional 34% is considered overweight.(1) Obesity-attributable medical expenditures related to the metabolic syndrome and other associated diseases reached $75 billion in the US in 2003.(2) Even with the billion-dollar diet industry, long-term weight loss results with dieting have been disappointing. Despite extensive research into possible targets for drug development aimed at the obesity epidemic, only surgical interventions including gastric bypass surgeries have shown persistent benefits.(3)

While tremendous progress has been made in the characterization of peripheral, vagal and hypothalamic mechanisms related to food intake and satiety in rodents,(4-9) it has only been recently that affective, reward and cognitive processes related to ingestive behavior and obesity have been studied in human subjects.(10, 11) Based on these studies, it has been suggested that overeating may be the result of an imbalance between vagally and endocrine mediated satiety mechanisms from the intestine with central reward circuits and prefrontal circuits involved in executive control of ingestive behavior. A similar shift between interoceptive, reward, and executive control mechanisms is seen in drug addiction where an altered interaction of these networks results in an enhanced reinforcing value of the drug and a distorted reward threshold.(10, 12)

A number of investigators have looked into the role of food related memories in obesity, by studying the impact of food images on brain activation.(13-15) Studies comparing lean and obese subjects have established differences in brain response to images of high calorie versus low calorie foods.(13, 14) It has also been demonstrated that brain activity changes in response to these food images after a subject consumes glucose versus water, and that this interaction between visual food cues and ingested nutrients differs in obese compared to lean groups.(15) A limitation to these studies is the fact that subjects can taste the difference between high and

low calorie foods and between glucose and water ingestion; thereby confounding the results by the subject's preconceived expectations based on what they are seeing, smelling and tasting. Non-caloric sweeteners stimulate the same sweet taste receptor as glucose and sucrose, thereby theoretically providing the same hedonic value without the calories.(16, 17) For this reason, they are an ideal means to study the specific effect of sucrose absorption on brain activation in obese and lean subjects.

In the current study, we aimed to test the following main hypotheses: i) Brain responses to the ingestion of a non-nutrient sweetened and a sucrose sweetened drink will be similar, presumably involving similar engagement of sweet taste receptors. ii) During visual food related cues, obese subjects will show greater affective and/or hedonic brain and behavioral responses to sucrose compared to lean controls. To test these hypotheses, we assessed brain responses using functional magnetic resonance imaging (fMRI) in lean and obese healthy female subjects during two drink conditions (a 300-calorie sucrose drink and an under 10-calorie non-nutrient sweetened beverage) that both stimulate sweet taste receptors.

Materials and Methods

Participant Selection

Twenty healthy lean and obese female participants were recruited from the clinical research unit of the UCLA Center for Neurobiology of Stress, from the UCLA Center for Human Nutrition, and from community advertisements. Subjects were between the ages of 18-40 years and were age-matched. The ten lean subjects had a body mass index (BMI) between 19-25 kg/m^2 and the ten obese subjects had a BMI between 30-37 kg/m^2. All subjects were right-handed, were regularly menstruating and were studied during the follicular phase (4-12 days after the first day of last menstrual period) given brain reward mechanisms vary with reproductive phase.(18)

Exclusion criteria were as follows: i) a history of any gastrointestinal surgery, psychiatric and neurologic disorders, or head trauma with loss of consciousness; ii) a past or current history of an eating disorder; iii) a current history of chronic pain; iv) being pregnant or breast-feeding; v) tobacco use of more than 5 cigarettes per month; vi) a history of excessive exercise; vii) postmenopausal status; viii) use of any medications/drugs that affect the central nervous system, gastrointestinal motility, autonomic activity or pain sensation within 4 weeks of enrollment; ix) a history of serious psychiatric, neurologic, cardiovascular, respiratory, or renal illnesses.

UCLA Office of Protection of Research Subjects (OPRS) approved the study protocol. All subjects provided written informed consent before participation.

Psychosocial Evaluations

Because psychological processes may influence gastrointestinal sensory and motor function as well as brain responses,(19) all subjects were evaluated using the MINI+5.0,(20) the Hospital Anxiety and Depression scale (HAD),(21) and the Spielberger State and Trait Anxiety Inventory (STAI).(22) The MINI+5.0 is a brief structured interview for the major Axis I psychiatric disorders in DSM-IV and ICD-10. The HAD scale is a measure of current anxiety and depression symptoms validated for non-psychiatric samples. The STAI is a 40-item self-report assessment that differentiates between state anxiety and trait anxiety.

Appetite Assessments

Taste, hunger, satiation, and satisfaction were assessed with two visual analog scales (VAS). The 10-point VAS for appetite examines taste and desire for specific food and has been shown to be reproducible and not influenced by prior diet standardization.(23) The 10-point Fullness

Questionnaire (FQ) is used to measure hunger and satisfaction and has been shown to correlate positively with the weight and caloric composition of foods, and negatively with palatability.(24)

Functional MRI Paradigm

This study was part of a larger study that used both resting state and task functional MRI to evaluate differences in obese and lean women after a sucrose drink versus a non-nutrient sweetened beverage. In this paper we are reporting only on the second part of the study (study 2) which focuses on the evoked brain responses to visual food cues after nutrient ingestion in obese and lean subjects.

A double-blind randomized crossover design was utilized over two separate days of functional MRI (fMRI) scanning, no less than 2 days apart. The details of the study protocol are shown in **Fig. 1**. Subjects fasted 6 hours prior to scan appointments, which occurred between 9:00 am and 11:00 am. The two scanning days were identical except the drink order was counterbalanced. At the start of each scan day, the subjects were given a synopsis of the study tests and placed in the scanner for a brief structural scan followed by two additional scans: a resting and a task-evoked functional food images scan.

The 15-minute functional food images scan had three 5 min functional RUNS (RUNS 1–3). During each RUN, 36 images (18 food, 18 neutral images of brick walls) were presented in random order. Subjects were instructed to focus their attention on the stimuli. Three images were shown for 4 s each followed by a 12 s dark screen with a central fixation cross. A total of 108 images were displayed (54 food, 54 scenery). In contrast to IAPS pictures, no standardized food images are currently available. As done by other investigators who have used food images,(13-15, 25) the images were selected from an internet repository. The wide array of

appetizing food images was chosen to represent a balance of food preferences. After the scans, subjects rated the images to ensure their palatability.

Ten minutes before both the resting scan and the functional food images scan, subjects consumed a 10 oz. beverage consisting of either a non-nutrient sweetened beverage (Diet Ocean Spray Cranberry Juice with 10 tsp of Truvia; <10 calories) or a sucrose beverage (Ocean Spray Cranberry Juice with 10 tsp of sugar; 300 calories). The beverages were designed to be similar in taste and sweetness. Pilot testing in 5 healthy individuals confirmed that the drinks could not be differentiated on the basis of taste. Subjects consumed both beverages on each scan day but the drink order was counterbalanced. The two beverages were given 25 minutes apart. Randomization was performed using Excel random number generator function. Subjects and investigators were blinded to the drink order. The beverage was presented in a non-descriptive container. Nose clips were applied to minimize olfactory influences and subjects were instructed to drink through a straw. As the study was not aimed at identifying the endocrine mediator(s) of the brain response to the test drinks, no measurements of plasma levels of glucose, insulin or other incretins were performed.

Subjects completed four FQs: a baseline FQ, a second FQ 10-minutes after the first beverage, a third FQ immediately prior to the second beverage, and a fourth FQ ten-minutes after the second beverage. A VAS for appetite questionnaires was completed ten-minutes after each beverage.

fMRI Acquisition and Preprocessing

MRI scanning was performed using a 1.5T MRI scanner (Siemens Sonata; Siemens, Erlangen, Germany). A high resolution structural image was acquired from each subject with a magnetization-prepared rapid gradient-echo (MP-RAGE) sequence, repetition time (TR) = 2200

ms, echo time (TE) = 4.38 ms, slice thickness = 1 mm, 176 slices, 256 x 256 voxel matrices, and 1^3 mm voxel size.

Food-related image stimuli were presented using E-Prime 2.0 Professional through MR compatible goggles. Functional blood oxygen-level dependent (BOLD) images were acquired with an echo-planar T2*-weighted imaging (EPI) sequence, TR = 2000 ms, TE = 45 ms, flip angle = 77°, slice thickness = 5 mm, 220 x 220 voxel matrices, and 3.4 x 3.4 x 5 mm voxel size.

Using SPM5 software (Welcome Department of Cognitive Neurology, London, UK), data were slice-time and motion corrected, spatially normalized to the MNI template using the structural images, and spatially smoothed at both 3 mm^3 and 8 mm^3 Gaussian kernel. The first two volumes were discarded to allow for stabilization of the magnetic field.

Data Analysis

Behavioral Analyses

Behavioral analyses were performed in PASW v17.0 (Chicago, IL). Group differences in STAI and HAD anxiety and depression ratings were evaluated by independent samples t-tests. Taste ratings were evaluated in a Group (lean; obese) x Condition (sucrose; non-nutrient sweetener) analyses of variance (ANOVA). Appetite ratings for satisfaction and hunger were evaluated in Group (lean; obese) x Condition (sucrose; non-nutrient sweetener) x Time (before ingestion; after ingestion) repeated measure ANOVAs. All data are given as mean ± standard error.

fMRI Analyses

To examine similarities between group brain responses to beverage consumption, we performed a region of interest (ROI) based conjunction analysis on the within group activation maps for the food-neutral image contrast obtained from applying a general linear model (GLM) in SPM5. Using the flexible factorial model option, subjects, groups (lean vs. obese), and

conditions (sucrose food images, sucrose neutral images, non-nutrient sweetener food images, and non-nutrient sweetener neutral images) were included as factors and the main effects of group and condition as well as their interaction were estimated. As no order effect was detected, order was not included in the final model. ROI analyses were conducted by applying a small volume correction (SVC) for the ROIs and significance was defined at a probability value less than 0.05 corrected using the family-wise error (FWE) algorithm. Anatomically based ROIs were selected a priori based on areas known to be involved in both hedonic and homeostatic networks (amygdala, hippocampus, hypothalamus, thalamus, and anterior and posterior insula). ROIs were created using the Wake Forest University PickAtlas toolbox in SPM5. To control for type I error inherent in testing multiple ROIs (n=6), significance of each region of interest was only considered after applying a modified Bonferroni procedure.(26)

Multiple regression and the ROIs described above were used to determine group (lean non-nutrient sweetener, lean sucrose, obese non-nutrient sweetener, obese sucrose) differences in the association between hunger scores and brain activity during the viewing of food images and viewing neutral images. Hunger scores were collected after the consumption of the second drink

A partial least squares (PLS) analysis was employed to identify distributed patterns of regions associated with viewing pleasant images of food during non-nutrient and sucrose beverage consumption in lean and obese women. PLS is a multivariate statistical technique considered to be more sensitive than standard univariate analyses of neuroimaging data such as SPM.(27, 28) PLS is analogous to principal components analysis (PCA), but the solutions can be restricted to the part of the covariance structure that is attributable to conditions or groups in an experimental design. Task PLS identifies experimental contrasts accounting for the maximum amount of variance in the data and the brain regions whose activity relates, as a whole, to these contrasts. In addition, a non-rotated PLS analysis was employed to examine group by condition interactions. The difference between a non-rotated and a task PLS analysis is that a priori

contrasts of interest are used in the non-rotated but not the task PLS. Contrasts representing group differences in response to food images in the high and low calorie conditions were entered into the analysis. PLS was implemented with freely available code (http://www.rotman-baycrest.on.ca) and performed on data spatially smoothed at 3mm$^{3.}$ Voxel reliability was determined using bootstrap estimation (500 samples). The ratio of the observed weight to the bootstrap standard error was calculated and voxels were considered reliable if the absolute value of the bootstrap ratio (BSR) exceeded 1.96. Clusters with a peak greater than 3.30 and greater than 60 voxels are reported.

Results

Patient Population

Clinical Variables

A total of 22 subjects were enrolled in the study (**Table 1**). The first two subjects were excluded from the GLM and PLS analyses due to suboptimal quality of our initial food and neutral images. Therefore, 20 subjects were used for the GLM and PLS analyses. The mean BMI of the lean group was 22.4 kg/m^2 (SE 0.5), and 32.9 kg/m^2 (SE 0.7) for the obese group ($p<0.05$). All obese and lean subjects' had similar STAI and HAD depression and anxiety ratings that were within the normal range. No subject had current depression or anxiety based on the MINI+ interview.

Appetite Measures

There was no difference between the sucrose and the non-nutrient sweetened drinks, in how their taste was rated by the two groups (**Supplemental Fig 1**). Beverage consumption significantly reduced hunger ratings ($p=0.005$), increased satisfaction ratings ($p<0.001$) and reduced desire for sweetness ($p<0.001$) across all subjects, without any differences between the two drinks. However, obese subjects rated the taste of both beverages significantly lower

than lean subjects (p=0.005), and obese women reported less satisfaction compared to lean subjects after both drinks, consistent with reduced subjective hedonic responses in the obese (p=0.016) (**Supplemental Table 1).**

Correlation of subjective sensations with brain responses

In both obese and lean subjects, a greater subjective hunger score on the FQ correlated with a greater engagement of the left posterior insula using ROI analyses (z= 3.70; p=0.045) when viewing food images after beverage consumption. Compared to lean subjects, obese subjects had a trend for greater correlation of the right anterior insula with the subjective feeling of hunger (z = 3.29; p= 0.074). No significant associations were detected for the other ROIs.

Brain responses related to test drinks in both lean and obese

Conjunction analyses of with in group activation maps for food– neutral image contrast demonstrated that in both groups, similar brain regions were engaged after ingestion of the two test drinks when viewing food images – neutral images. Specifically, there was significant engagement of bilateral amygdala, bilateral hippocampus, and bilateral thalamus, and right anterior insula, consistent with engagement of a network of brain regions related to affect, memory and interoception (**Table 2**).

Brain responses to food cues and differences between obese and lean subjects

A multivariate analysis (task PLS) was used to identify a network of interactive regions that were engaged differently between groups during the four conditions. In both subject groups, the viewing of food images was associated with activation of a network that included the left insula,

bilateral pregenual cingulate, bilateral amygdala, and left hippocampus (**Table 3**). This network accounted for 41% of the crossblock variance ($p<0.001$), and was engaged to a greater extent in the obese compared to lean subjects (**Fig. 2**).

Using a non-rotated PLS, we found an additional network that was more engaged by obese subjects only after the high calorie sucrose beverage compared to all other group/conditions. This network included bilateral anterior insula, right anterior cingulate cortex, bilateral amygdala, the left hippocampus, and the visual cortex (**Table 3**). This network accounted for 27% of the crossblock variance ($p=0.028$) (**Fig. 3**).

Discussion

The main findings of the study are: 1) Similar brain regions were engaged after ingestion of either sucrose or a non-nutrient sweetener while viewing hedonic food images. 2) In both groups, regardless of drink type, the viewing of pleasant food images was associated with brain networks involving interoceptive, affective and cognitive brain regions. 3) While all subjects rated the taste and the satisfaction of the two drinks similarly, obese women rated both drinks as less tasteful and satisfying than lean women, consistent with a reduced hedonic response to the drinks. 4) This reduced behavioral hedonic response in the obese was associated with greater responses in affective and memory related brain regions to both viewing of food images, and to the ingestion of the sucrose drink. Even though other interpretations are possible, these results suggest that in obese subjects, gut derived sucrose related signaling, presumably transmitted to the brain via vagal afferent and/or endocrine pathways, generates less hedonic effects than recalling memories of pleasant food in response to visual cues. As the latter response appears to be reinforced by sucrose ingestion, it may be a mechanism whereby sucrose ingestion perpetuates the craving for more sweets.

Similarities in brain responses to sucrose and non-nutrient sweetener

In the combined sample, similar brain regions were engaged after ingestion of the two test drinks while viewing hedonic food images. The regions included the bilateral amygdala, bilateral hippocampus, bilateral thalamus, and right anterior insula. These findings are consistent with the multidimensional encoding of interoceptive, affective, memory related and cognitive aspects of the experience of a sweet test meal, as previously reported.(23) Non-nutrient sweeteners bind to lingual taste receptors with equal if not greater affinity as sucrose.(16, 17) The similarity of the brain response between the two drinks suggests that the interoceptive, presumably vagally mediated input to the brain results primarily from lingual and possibly intestinal sweet taste receptor activation, and does not require other encoding mechanisms, which require glucose absorption and interaction with glucose sensing mechanisms in the portal vein, the pancreas and the brain (hypothalamus, nucleus tract solitarius).(29, 30) However, since blood glucose or insulin levels were not assessed in the current study, the possible role of such endocrine signals in the observed brain responses cannot be answered directly.

Differences in subjective ratings of the test meal by obese and lean subjects

Despite the difference in caloric content between the two drinks, we found that subjects rated the taste of the sucrose and the non-nutrient sweetened beverage similarly, suggesting similar activation of lingual sweet taste receptors by the two drinks. Sweet taste perception of both non-nutrient sweeteners and sucrose is peripherally mediated by tongue heteromic T1R2/T1R3 sweet taste receptors. The sensory information is then transmitted by cranial nerves VII, IX and X to the nucleus tract solitarius, and to the human gustatory cortex within the anterior insula.(31)

Previous studies have shown artificial sweeteners bind to these taste receptors with equal if not greater affinity compared to sucrose.(16, 17, 32) For example, it has been reported that both sucrose and sucralose applied lingually active functionally connected primary taste pathways and related brain regions.(31)

Obese subjects rated the taste of both beverages lower than lean subjects, and reported less satisfaction after consuming the beverages compared to lean subjects, consistent with a reduction in the hedonic aspect associated with ingestion of a sweet drink. The subjective perception of sweet taste is a multidimensional experience and reflects the modulation of anterior insula activity by inputs from interoceptive, affective, reward, and prefrontal/orbitofrontal inputs.(21) Taste perception includes the assessment of taste quality, hedonic "liking" and the incentive motivational component "wanting".(31, 33) Reduced satisfaction to actual food ingestion ("liking") despite greater engagement of hedonic circuits during expectation of food intake ("wanting"), has been proposed as a mechanism underlying food addiction.(34) This pattern is similar to drug addiction where craving for the drug is amplified, while actual satisfaction after drug use is reduced.(34) In our study, the subjects were shown palatable images of food after beverage consumption and in between behavioral measure assessment. Altogether, these findings imply that in obese subjects, gut derived signaling generates less hedonic effects than recalling memories of food in response to visual cues.

Recall of food-related memories by visual stimuli and interaction with interoceptive stimuli

It has long been known that amnesic patients readily eat a second meal offered immediately after a full meal(11) suggesting that memory recall of food related experiences are as equally significant as caloric need in the decision to eat. Representations of food-related experiences

are generated in networks involving the prefrontal and orbitofrontal cortices, anterior insula, amygdala, hippocampus and reward pathways.(8, 33) Consistent with previous reports, we found that in both lean and obese subjects, regardless of drink type (and possibly differences in endocrine mediators), the viewing of pleasant food images was associated with activation of interoceptive, affective and memory related brain regions.(35, 36) The fact that we did not see a difference in BOLD response to the two drinks in the hypothalamus in either group or condition comparison may be related to the small sample size.

Obese subjects have an exaggerated response to food images after a sweetened drink

While similar brain regions were engaged with both the sucrose and the non-nutrient sweetened beverage in the combined lean and obese groups, there were differences in brain responses when the obese women were compared to the lean group. When obese subjects viewed food images after the sucrose beverage (but not the non-nutrient sweetened drink), a network including the anterior insula, anterior cingulate cortex, right lateral amygdala, right hippocampus, and the visual cortex was more engaged compared to lean subjects. Similarly, Rothemund et al(37) found that high-calorie food images yielded BMI-dependent activations in regions associated with taste information processing (anterior insula and lateral orbitofrontal cortex), motivation (orbitofrontal cortex), and emotion and memory functions (posterior cingulate). These findings are consistent with the concept that viewing food images produces a brain response involving recall of previous food experiences, and that this response is exaggerated in obese subjects.

While both obese and lean subjects showed the expected positive correlation between the subjective feeling of hunger with the engagement of insular cortex when viewing food images postprandially,(38, 39) obese subjects had a greater correlation of the right anterior insula with the subjective feeling of hunger. This suggests a greater modulation in the obese subjects of

this brain region by emotional and reward pathways when craving for food. Anterior insula activation has also been demonstrated in association with the conscious feeling of urge in drug addiction.(12)

Our findings suggest that in obesity, additional interoceptive inputs other than that generated by lingual/intestinal sweet taste receptor activation may play a role in the associated brain response to a sweetened beverage in the context of viewing food cues. In addition to sweet taste receptor activation on intestinal enteroendocrine cells, glucose-sensing mechanisms have been described in the pancreas, portal vein, hypothalamus and the nucleus of the solitary tract.(29, 30, 40-42) While group differences between lean and obese subjects in peripheral glucose, insulin, or incretin levels after sucrose intake may play a role, the current study was not designed to identify such differences.

As an alternative to peripheral differences in glucose sensing, central differences between obese and lean populations might also explain our findings. In the drug addiction model, as addiction increases, stimuli within the environment that are associated with drug use (cigarettes, bottles of alcohol, drug paraphernalia) become powerful reinforcing incentives to drive ongoing drug use. This suggests the interoceptive cortex has a central role in conscious cue-induced urges by encoding a representation of the salient effects of drug use that become activated when an addicted person is exposed to drug cues. It is believed that the amygdala and hippocampus are also involved in conditioning to addictive substances and relative cues in addiction.(43) Similarly, in obesity an addiction to sucrose might prompt increased engagement of this salience network when viewing images of palatable food after ingesting sucrose.

Limitations

A potential limitation to our study is the fact that the volumes of the food were the same for

obese and lean, possibly contributing to the finding that obese subjects were less satiated – obese women might require a larger volume to feel full. However, the absence of a statistical differences in hunger ratings pre and post beverage consumption, suggest that volume did not play a significant role in the satiety measures. Similarly, group differences in gastric emptying may have contributed to the observed differences. The findings in rodent studies(44) that no difference in gastric emptying after infusion of sucrose or an artificially sweetener is observed, argues against a group difference. Another potential limitation is the fact that no direct measurements of glucose, insulin and incretin levels at baseline and following the test meal were performed. While an interesting target for future mechanistic studies, such measurements were not essential to address the main hypotheses of the current study related to group differences in behavioral and brain responses between lean and obese subjects.

Summary and possible clinical implications

In summary, we found several obesity-related differences in behavioral and brain responses to sucrose versus a non-nutrient sweetened beverage. Obese women verbally reported a reduced hedonic behavioral response to either sweetened beverage, yet they demonstrated a greater hedonic brain response particularly after sucrose ingestion. This increased brain response is driven more by recalling memories of food experiences in response to visual cues than by lingual and gut derived signaling. Despite the extensive literature on obesity related changes in peripheral satiety mechanisms,(6, 7) the findings of the current study are most consistent with a difference in central modulation of ingestive behavior in the obese, and are suggestive of food addiction.

Tables

	Lean Mean (SE) n=10	Obese Mean (SE) n=10
Age (yr)	24.60 (1.33)	26.50 (1.64)
BMI (kg/m^2)	22.40 (0.50)	32.91 (0.74)
HAD Depression	1.20 (0.39)	2.20 (0.44)
HAD Anxiety	5.70 (1.25)	4.93 (1.06)
STAI	49.20 (3.56)	46.67 (2.82)

Table 1. Demographics and Baseline Characteristics

ROI	Coordinate (x,y,z)	Cluster value (Ke)	P value (FWE)	Z score
R Amygdala	(-24, -6, -14)	117	0.020	3.24
L Amygdala	(24, -6, -16)	136	0.007	3.60
R Hippocampus	(-28, -36, 2)	32	0.009	3.52
L Hippocampus	(26, -30, -6)	56	0.002	4.00
R Thalamus	(22, -26, -2)	364	0.007	3.98
L Thalamus	(-20, -30, 0)	281	0.004	4.14
R Anterior insula	(-24, -6, -14)	117	0.020	3.24
L Anterior insula	(-26, 30, -8)	18	0.321	2.68

Table 2. Similar brain regions were engaged when looking at images of food after ingestion of both sucrose and the non-nutrient sweetener beverages in lean and obese subjects, using ROI conjunction analysis, as described in Methods. The results for the following ROIs were not significant: posterior insula, hypothalamus.

Analysis	Region	Coordinate (x,y,z)	Cluster value (voxels)	Approx. P value	Bootstrap Ratio
TASK PLS	L BA17/18/19	(-44, -84, 2)	5281	0	9.2475
Food > Neutral	R BA18/19/39	(42, -78, 8)	5986	0	8.2686
	L Cerebellum	(-18, -34, -46)	71	0	5.5753
	R Cerebellum	(24, -34, -40)	100	0	5.5712
	L BA24	(-4, 4, 28)	66	0	5.5022
	L BA47	(-30, 30, -12)	270	0	5.3495
	R amygdala/PHG/ basal ganglia	(28, -2, -14)	393	0	5.0527
	L Insula	(-38, -10, 10)	192	0	5.0482
	L BA6	(32, -8, 56)	89	0	5.0031
	L amygdala/PHG/ hippocampus	(-18, -8, -18)	324	0	4.9989
	R Caudate	(34, -36, -2)	64	0	4.9537
	R BA9/46	(52, 4, 36)	493	0	4.544
	L BA9/46	(-58, 6, 30)	252	0	4.4831
	R Insula	(40, -6, 4)	83	0	4.2976
	L/R BA6/32	(-4, 12, 52)	208	0	4.181
	R Thalamus	(4, -40, -12)	166	0	4.1239
	L BA40	(-42, -42, 42)	219	0	4.0752
	R BA6	(0, 14, 66)	125	0.0001	4.0478
	L/R Midbrain	(2, -20, -14)	92	0.0001	4.0306
	R Cerebellum	(16, -54, -46)	124	0.0001	4.0259
	L BA17/18	(-12, -94, 2)	97	0.0001	4.0206
	L Insula	(-28, -30, 20)	72	0.0001	3.9012

	L/R BA8/32	(-10, 32, 42)	173	0.0001	3.8883
	L BA24/32	(-6, 32, 20)	137	0.0003	3.6452
	R BA45/46	(46, 32, 4)	92	0.0003	3.6238
	L Cerebellum	(-28, -72, -48)	97	0.0003	3.6037
	R BA32	(14, 20, 30)	95	0.0003	3.5874
	L Cerebellum	(-6, -80, -28)	72	0.0009	3.3164
NON-ROTATED	R BA 18/19	(30, -80, 24)	3200	0	10.5634
TASK PLS	L BA 18/19	(-44, -84, 2)	2314	0	8.4669
Food > Neutral	L BA 40	(-48, -36, 48)	139	0	7.0144
	R BA 7	(30, -62, 62)	79	0	6.8714
	R Cerebellum	(18, -42, -44)	103	0	6.6146
	R Insula	(52, 14, 0)	209	0	6.5611
	L BA 7	(-22, -64, 42)	364	0	6.5473
	R BA 32	(12, 12, 40)	211	0	6.336
	R BA 9	(50, 4, 42)	259	0	6.0134
	R BA 20	(42, -18, -26)	119	0	5.906
	L BA 6	(-4, 12, 54)	279	0	5.8971
	L PHG/Hippocampus	(-20, -30, -8)	181	0	5.8168
	L Insula	(-40, 8, 2)	131	0	5.6259
	R BA 6	(32, -8, 54)	94	0	5.32
	L Amygdala	(-32, 0, -24)	136	0	4.843
	R BA 2/3	(54, -20, 30)	73	0	4.351
	R Thalamus	(4, -24, 0)	71	0	4.3483
	R Amygdala	(18, -2, -8)	96	0	4.2853
	L BA 6	(-44, 0, 34)	134	0.0001	3.9377
	L BA 47	(-40, 24, -10)	103	0.0002	3.7485

	L BA 4	(-36, -22, 36)	71	0.0005	3.4908

Table 3: Network analyses revealed a network of regions associated with viewing of food images in both lean and obese groups that accounted for 41% of the crossblock variance ($p<.001$) and was more engaged in the obese subjects (Task PLS) and an additional network more engaged by obese subjects only after consumption of the high calorie sucrose beverage that accounted for 27% of the crossblock variance ($p=0.028$) (Non Rotated PLS). Analyses detailed in Methods.

Figures

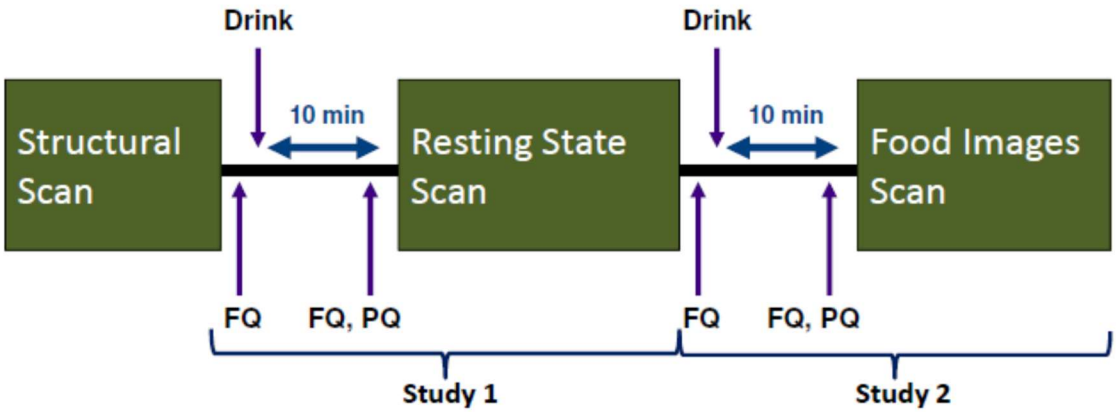


Figure. 1. Study protocol. On the scanning day, subjects underwent three types of scans; a brief structural scan, a resting state scan, and a food images scan. 10 minutes before both the resting state and food images scans, subjects received a fullness questionnaire (FQ) and either a non-nutrient sweetened drink or a sucrose beverage. Immediately prior to scanning, subjects were given another FQ as well as a palatability questionnaire (PQ). This scanning day was repeated with the order of the drinks counterbalanced. The resting state scan along with the drink and questionnaires given prior to the scan were analyzed together as study 1 (results

presented in a different paper). The food images scan and accompanying drink and questionnaires (study 2) are analyzed in this paper.

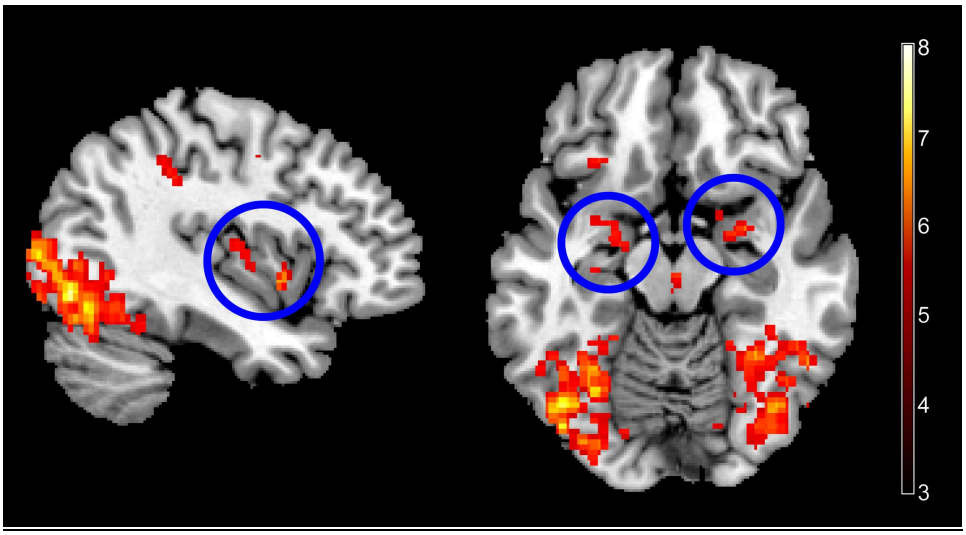


Figure 2. Obese show greater engagement of brain network including posterior INS (left panel) and bilateral amygdala (right panel) to viewing food images, using a multivariate fMRI analysis approach, as described in Methods. A blue circle marks respective brain regions**.**

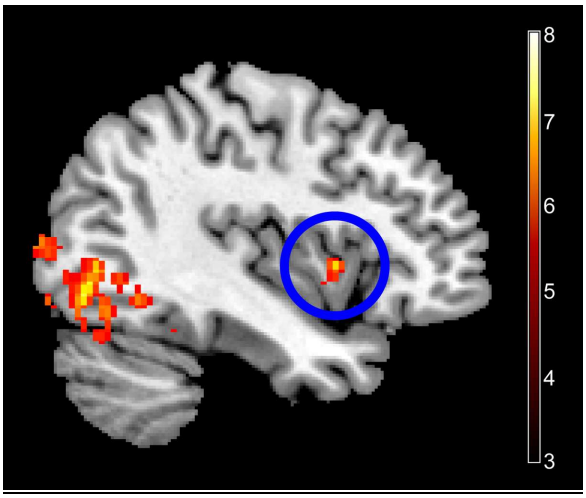


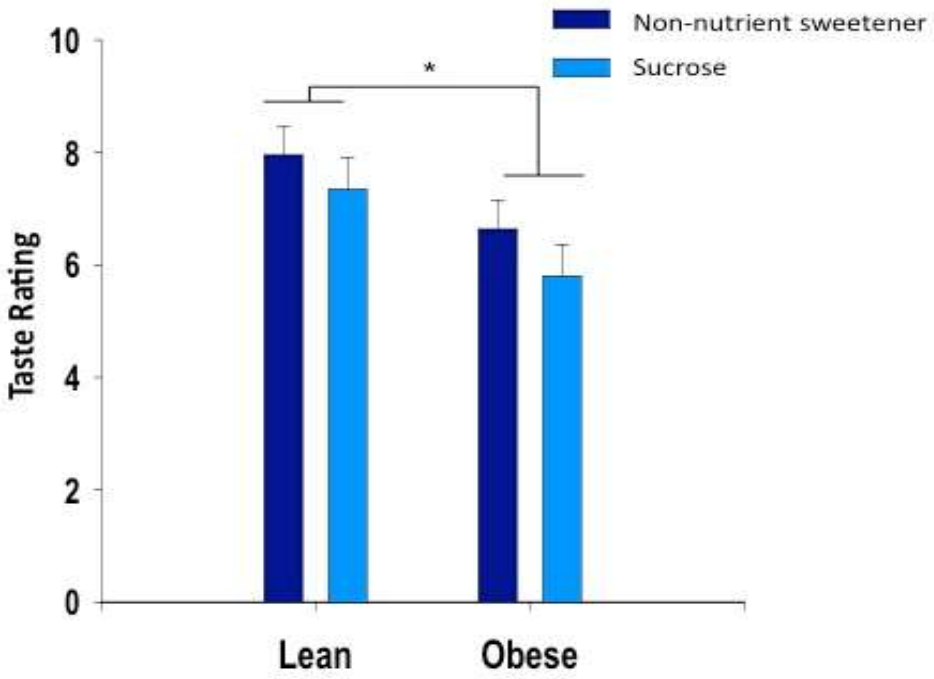
Figure 3 Obese subjects had greater engagement of a network including the anterior INS (blue circle) following the high calorie sucrose beverage, using multivariate fMRI analysis approach, as described in Methods. ($p<.001$)

Supplemental Figures and Tables

	Lean Subjects Mean (SE)		**Obese Subjects Mean (SE)**	
	Low Calorie	**High Calorie**	**Low Calorie**	**High Calorie**
Taste	8.18(.39)	7.73(.50)	6.86(.61)*	5.73(.71)*
Hunger:				
Baseline	5.40(.61)	5.35(.85)	5.8(.49)	5.50(.44)
After 2nd Drink	3.28(.98)	3.55(.76)	4.75(.60)	4.20(.61)
Satisfaction:				
Baseline	1.50(.40)	1.60(.31)	2.30(.55)	2.20(.52)
After 2nd Drink	4.44(.85)	4.80(.84)	3.70(.44)	3.95(.48)
Desire for Sweetness:				
Baseline	4.85(.71)	3.45(1.02)	5.35(.65)	4.40(.73)
After 2nd Drink	6.94(.85)	7.95(.50)	7.25(.59)	7.10(.53)

*$p<0.05$ between group comparisons

Supplemental Table 1. Taste and appetite ratings at baseline and after the second beverage consumption. Subjective ratings were quantified using a 10-point VAS, as described in the methods.



Supplemental Figure 1. Comparing taste ratings of sucrose and non-nutrient sweetened beverages between obese and lean subjects (*p>0.05). Subjective taste ratings were quantified using a 10-point VAS, from “bad = 0” to “good = 10”.

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CHAPTER THREE

Background: Using Resting MRI to Study Obesity

Overview

A major limitation of applying functional imaging techniques to study the brain's response to food intake has been the traditional need for phasic on/off paradigms to study hemodynamic (BOLD) responses. This paradigm has not been feasible for the study of satiety signals. However, it is now appreciated that a wealth of information exists in the fluctuations in the BOLD signal that are seen in the brain even when the subject is at rest and not actively engaged in discrete tasks, ushering new approaches to fMRI design and analysis.

Oscillatory Dynamics of the Resting Brain

One such approach is to study the frequency features of intrinsic brain activity. It has been suggested that specific frequency band oscillations may correlate with different regions (i.e. cortical or sub-cortical), based on communication strength (local vs long term signaling) and that an alteration of frequency power may reflect a change in regional processing (1, 2). Frequency bands have also been associated with specific functions. For example, brain regions involved with pain processing have been shown to have higher frequency oscillations (3, 4).

Patients with cognitive disorders typically display altered frequency patterns (5, 6), and analysis of the brain's oscillatory dynamics can provide novel insights into the effect of both clinical conditions and physiological manipulations on brain processes. For example, altering serotonin function by acute tryptophan depletion has been shown to increase high frequency power in brainstem raphe nuclei as well as change raphe connectivity patterns (7). The identification of altered region-specific frequency shifting can also identify regions of interest for further examination with other resting state analysis techniques (7, 8).

The determination of varying oscillatory dynamics in and of itself is quite novel, can offer insights, but the physiological underpinnings are less understood. One potential interpretation

of frequency power is that it relates to neuronal activity, with increased frequency power indicating reduced neural firing (9, 10).

Functional Connectivity

An increasingly popular approach to studying the resting brain involves the assessment of intrinsic functional connectivity among brain regions, typically using seed correlation analysis or independent component analysis to test the strength of the connection between one region with the rest of the brain. Recent studies have shown altered intrinsic functional connectivity during resting conditions in various clinical conditions (11, 12). Furthermore, connectivity studies going beyond the standard resting protocol have demonstrated that cognitive and physiological manipulations (including ingestion of alcohol and marijuana) can impact the intrinsic connectivity of networks in healthy subjects(13, 14).

Obesity and The Resting Brain

Neuroimaging studies on resting state and obesity are limited, but have provided several insights. For one, they have highlighted the parallels between drug addiction and obesity suggesting that addictive and natural reinforcement from food depends on the engagement of brain regions associated with reward(15). Kilpatrick et al reported that ingestion of a carbohydrate test meal was associated with altered connectivity and oscillatory dynamics in both lean and obese women(8). Nutrient ingestion was associated with a shift to high frequencies in the vagal nucleus of the solitary tract (NTS), suggesting increased neural activity in a brainstem region that receives vagal afferents from the gut and is associated with homeostatic processes in relation to food intake (Figure 1).

Although this shift was seen in both lean and obese women, the groups differed in the relationship of NTS high frequency power to subjective hunger ratings and functional connectivity patterns. For lean but not obese women, decreased hunger ratings following beverage consumption was associated with greater high frequency in the NTS. These results suggest a connection between activity in homeostatic input regions and self-reported hunger in lean women that is less present in obese women. The NTS was shown to interact differently with other brain regions such as the thalamus and putamen(8).

For obese women, but not lean, increased high frequency power in the NTS was associated with increased connectivity between the NTS and these reward and motivation-related brain regions, suggesting enhanced reward/motivation effects of food intake in obese individuals. While there seems to be differences in the intrinsic oscillation patterns of the obese vs lean brain, and these patterns seem to be impacted by ingestive behavior, the role of something like a hormone in altering these patterns is in need of further study.

Figures

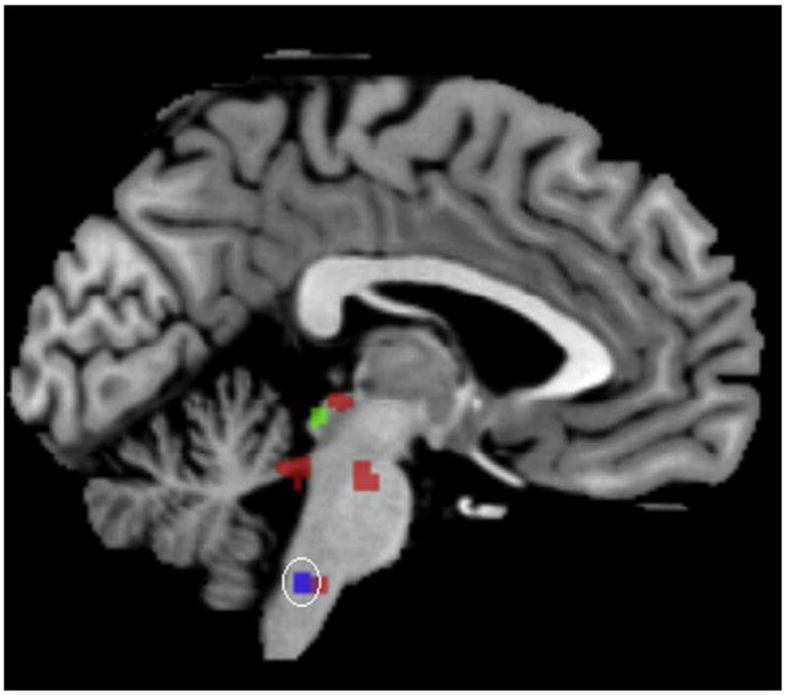

Fig. 1 Activity in brainstem regions demonstrated sucrose-related redistribution of frequency power. *Red*: reduced LF/MF frequency power after ingestion of a high-sucrose beverage compared with a low-sucrose beverage in both lean and obese women. *Blue*: greater HF frequency power after ingestion of a high-sucrose beverage compared with a low-sucrose beverage in both lean and obese women. *Green*: greater HF frequency power in lean compared with obese women regardless of drink condition. NTS is shown *circled*. frequency for lean women compared with obese women

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CHAPTER FOUR

Manuscript: Altered Functional Connectivity within the Central Reward Network in Overweight and Obese Women *(published Nutrition & Diabetes, 2015)*

Abstract

Background/Objectives: Neuroimaging studies in obese subjects have identified abnormal activation of key regions of central reward circuits, including the nucleus accumbens (NAcc), in response to food related stimuli. We aimed to examine if women with elevated body mass index (BMI) show structural, and resting state (RS) functional connectivity alterations within regions of the reward network. **Subjects/Methods**: 50 healthy, premenopausal women, 19 overweight and obese (high BMI=26-38 kg/m^2) and 31 lean (BMI=19-25 kg/m^2) were selected from UCLA's Oppenheimer Center for Neurobiology of Stress database. Structural and RS functional scans were collected. Group differences in grey matter volume (GMV) of the NAcc, oscillation dynamics of intrinsic brain activity, and functional connectivity of the NAcc to regions within the reward network were examined. **Results**: GMV of left NAcc was significantly greater in the high BMI group than in the lean group (p=0.031). Altered frequency distributions were observed in women with high BMI compared to lean in the left NAcc (p=.009) in a medium frequency band and in bilateral anterior cingulate cortex (ACC) (p=.014, <.001) and ventro-medial prefrontal cortex (vmPFC) (p=.034, <.001) in a high frequency band. Subjects with high BMI had greater connectivity of the left NAcc with bilateral ACC (p=.024) and right vmPFC (.032) in a medium frequency band and with the left ACC (p=.03) in a high frequency band. **Conclusions**: Overweight and obese women in the absence of food related stimuli show significant structural and functional alterations within regions of reward-related brain networks, which may play a role in altered ingestive behaviors.

Introduction

In the lean individual, food intake and body weight are under tight homeostatic regulation.[1] Bidirectional signaling between the gut, adipose tissue and the brain acts as an energy-balancing circuit assuring a stable body weight even in the presence of fluctuations of food intake and physical activity. However, in obesity relative changes in the inputs to this circuit have to be considered which might disrupt or off-set this balance.[2] One important input comes from the mesolimbic dopamine system which drives food related behaviors and plays a crucial role in the complex interactions between hedonia, reinforcement, motivation, incentive salience, and reward prediction.[3, 4] From an evolutionary standpoint, this system optimizes survival in a world of limited food supply. [5] However, when highly palatable food is readily available, increasingly hedonically driven consumption can lead to an altered state of homeostasis.[2] Under these circumstances, the ability to resist the urge to eat is no longer solely dependent on metabolically driven satiety signals, but becomes dependent on the inhibitory effects of prefrontal control mechanisms.

Neuroimaging studies in human subjects have highlighted the parallels between drug addiction and a subset of obesity,[6] suggesting that addictive and natural reinforcement from food is dependent on the engagement of brain regions associated with reward.[4] Food addiction has been implicated as an important factor in the pathophysiology of obesity in about 11% of overweight and obese women.[7] For example, alterations in dopaminergic input from the ventral tegmental area (VTA) and substantia nigra to the nucleus accumbens (NAcc) can lead to a dysregulation of motivational and reward properties associated with food intake.[8] In fact, individual differences in NAcc responses to contextual food cues (including visual, auditory and olfactory) may be one of the main reasons for obese individuals to show addiction like behaviors resulting in weight gain.[9] The majority of insights about brain alterations in food addiction come

from PET studies using dopamine receptor ligands,[10] and tasked based fMRI studies that look at hemodynamic (BOLD) signals while subjects undergo a specific task, such as viewing images of food.[11] Studies have also reported obesity related differences in brain structure. For example, BMI has been shown to be positively correlated with grey matter volume (GMV) in the medial orbitofrontal cortex (OFC), hypothalamus (HYP), and left putamen.[12] In addition, several studies have identified increases in GMV of the NAcc in obese subjects compared to lean subjects[12, 13] as well as in subjects who had increased eating behaviors but similar BMI compared to control subjects.[13] When viewed together, these results suggest that the NAcc is not only associated with evaluating motivationally salient stimuli (food) and in evaluating its subjective value, but that chronically altered ingestive behavior is associated with neuroplastic structural changes in the NAcc.[12]

There is limited information about obesity related differences in spontaneous intrinsic BOLD oscillations in the brain (resting-state [RS] fMRI). Several RS analytic techniques such as fractional amplitude of low-frequency fluctuation (fALFF) (which computes regional power spectrum intensity of spontaneous brain oscillations)[14] and intrinsic functional connectivity have been used to identify disease-related alterations associated with specific brain regions. Such techniques have been used to examine altered neuro-circuitry in obesity.[15-17]

Based on previous reports on alterations of the extended reward network in obesity, we aimed to test the following hypotheses in healthy women with and without elevated BMI values: Subjects with elevated BMIs 1) have larger NAcc volumes compared to lean controls, reflecting increased engagement of the reward system and/or secondary neuroplastic effects of obesity on the brain; 2) show alterations in intrinsic oscillatory dynamics of BOLD signal for regions of the extended reward network, including the NAcc; and 3) show increased NAcc functional connectivity within the reward network compared to lean controls.

Methods and Materials

Participant Selection

Fifty healthy women aged 18-40 years old were recruited through the University of California, Los Angeles, and community using advertisements. The sample included 31 lean women (mean age 25.42 years old, SD=5.86; Body Mass Index [BMI] 19-25 kg/m^2) and 19 overweight or obese women (mean age 27.05 years old; SD=7.03; BMI 26-38 kg/m^2). All subjects were right-handed, pre-menopausal, and classified as healthy after a clinical assessment that included a modified Mini-International Neuropsychiatric

Interview Plus 5.0,[18] a brief structured interview for major Axis I psychiatric disorders in DSM-IV[19] and ICD-10,[20] and the Hospital Anxiety and Depression (HAD) questionnaire.[21]

The subjects provided written informed consent and all procedures were reviewed and approved by the UCLA Medical Institutional Review Board. Further exclusion criteria included pregnancy, substance abuse, tobacco dependence, psychiatric illness, diabetes, and any digestive or eating disorders such as anorexia or bulimia nervosa. Subjects were also excluded if they were currently on medication such as analgesics or antidepressants. Subjects who had undergone any obesity-reduction surgery were also excluded.

Structural and Resting State data acquisition

MRI images were acquired on a Siemens 3 Tesla Trio scanner. Structural scans included a standard T1-weighted magnetization-prepared rapid acquisition gradient echo (MP-RAGE) scan which was acquired with the following parameters: TE = 3.26ms, TR = 2200ms, slices= 176, slice thickness = 1.0mm, voxel size = 1x1x1mm, with the exception of three obese subjects' whose structural images were collected with the following parameters: TE = 3ms, TR = 2000ms, slices = 160, slice thickness = 1mm, voxel size = 1x1x1mm.

Functional MRI RS scans between 8m6s and 10m6s in length were acquired with an echo planar sequence with the following parameters: TE = 28ms, TR = 2000ms, scan duration = 8m6s – 10m6s, flip angel = 77 degrees, FOV = 220mm, slices = 40, slice thickness = 4.0mm, slices were obtained with whole-brain coverage.

Structural MRI image processing and analysis

Outlines around the NAcc were manually created with the help of the intensity contour function within FSLview (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl.[22, 23] First slices were selected where the anterior NAcc is most visible. In order to determine the superior border of the NAcc, an oblique line was drawn from the inferior most tip of the lateral ventricle (where it meets the caudate) to the inferior most medial tip of the putamen. Then the inferior border of the NAcc was determined using the contour function and with the help of determining the position of the caudate (http://neuromorphometrics.org:8080/Seg/html/segmentation/accumbens_area.html). The LONI (Laboratory of Neuroimaging) pipeline (http://pipeline.loni.usc.edu/), a graphical workflow environment was utilized to obtain total brain volumes (TBV) for all subjects. The NAcc ROI values were then aggregated and entered in a general linear model (GLM) in SPSS while controlling for age and TBV in order to compare the NAcc volumes in the two groups (obese and overweight compared to lean female subjects).

Resting- state MRI image preprocessing

Image processing and data analysis were performed using Statistical Parametric Mapping 8 (SPM8) software (Wellcome Department of Cognitive Neurology, London). Processing was done through the SPM toolbox, Data Processing Assistant for RestingState fMRI (DPARSF),[24] where data were slice-time and motion corrected. Nuisance covariate regression was performed to minimize physiological noise using six head motion parameters, white matter signal and corticospinal fluid signal. Data were spatially normalized to the Montreal Neurological Institute (MNI) template using structural scans. Spatial smoothing with a $4mm^3$ Gaussian kernel occurred after calculation of frequency and connectivity maps.[24]

Frequency analysis

Although frequency analyses of resting state scan data typically compute power with a 0.01-0.10 Hz band, the frequency spectrum can be further subdivided to better reflect the neural origin of their sources.[14] For each resting state scan, the BOLD signal time-course data of each voxel was transformed to the frequency domain and was subdivided into slow-5 (0.01-0.027 Hz), slow-4 (0.027-0.073 Hz) and slow-3 (0.073-.198 Hz) frequency bands thought to represent different neuronal oscillation classes.[25, 26] These bands will be referred to low frequency (LF), medium frequency (MF) and high frequency (HF) respectively. Relative power within each of the three bands was computed for each voxel in the brain using fALFF and normalized to the mean.[14] This technique involves summing the oscillatory amplitudes across a particular frequency range (i.e. 0.027-0.073 Hz) then dividing by the amplitude sum across a more inclusive range (i.e. 0-.25 Hz), thereby assessing the ratio of power for a particular frequency band to the power of the entire frequency range. A grey matter mask was applied to restrict analysis to grey matter regions. For the results of the frequency band analyzes, we focused on the LF and MF bands only as the HF band can contain noise (possibly due to interference from physiologic measures).

The GLM in SPM8 was used to compare group data (overweight and obese; lean) x band (LF; MF), with age included as a covariate. Using a region of interest (ROI) approach,[27] group contrasts (overweight and obese group versus lean group) were performed for each band to identify regions with altered frequency power distributions. Anatomically-based ROIs were created using the Wake Forest University PickAtlas toolbox in SPM8. Regions were chosen based on involvement within the extended reward network as upregulation of reward circuits that drive ingestive behavior include brain regions concerned with reward, salience, central

autonomic, and cortical inhibition (prefrontal control). The brain regions included NAcc, hippocampus (HIPP), OFC, ventromedial prefrontal cortex (vmPFC), anterior cingulate cortex (ACC), amygdala (AMYG), insula (INS), and regions of the striatum (caudate, putamen, pallidum).[4, 5, 8] Contrast images were thresholded at p=.001, uncorrected and small volume correction was employed to determine significance of ROI based on p<.05, corrected for family wise error (FWE) rate. False discovery rate (FDR) was applied to control for the type I error inherent in testing multiple ROIs.[28, 29]

Seed-based Functional Connectivity Analysis

Altered frequency power distribution can be accompanied by altered functional connectivity;[30-32] thus the functional connectivity of the regions identified in the above analysis was examined, focusing particularly on the region in the left NAcc. Using MarsBar,[33] a seed was defined for the NAcc using results of the frequency analysis; thus the NAcc seed was confined to the portion of the NAcc demonstrating altered frequency distribution. For readability, the functionally defined NAcc cluster is sometimes simply referred to as the NAcc in results and discussion sections. In addition, the other reward-related regions with significant group differences in frequency power were used as ROIs in the functional connectivity analysis (ACC, vmPFC). Band specific fisher transformed maps of the bivariate correlation between seed ROI timecourse and all other voxels were created using DPARSF. Band-specific functional connectivity in overweight and obese versus lean women was compared using an ROI approach similar to other studies,[34] and a two-sample t-test in SPM8 using age as a covariate. Significance was determined at $p<.05$ FWE corrected and ROIs were further FDR corrected for multiple comparisons.[28, 29] Correlations between brain measures (structural, frequency power and functional connectivity) and BMI were conducted in SPSS.[35]

Results

Demographic and Clinical Characteristics

The subject groups showed no statistically significant difference in age or any clinical measure (**Supplementary Table 1**). BMI for the lean group ranged from 19.5225.09 kg/m^2 with an average of 22.32 kg/m^2. BMI for the overweight/obese group ranged from 25.88-37.56 kg/m^2 with an average of 31.83 kg/m^2. With all values being within the normal range, the two groups also did not differ in symptom levels of anxiety (F=.361, p=.553), depression (F=2.301, p=.139).

Structural analysis (NAcc Volumes)

After controlling for age and total brain volume, the high BMI group had larger bilateral NAcc volumes compared to the lean group (**Table 1**), even though this difference only reached statistical significance for the left hemisphere (F=4.936 p=.031). The left NAcc volume showed a significant correlation with BMI over all subjects (r= .351; p=.013; **Figure 1**).

fALFF group comparison

The fALFF results for the MF and LF bands are summarized in **Table 2.** When comparing the high BMI group with the lean group, the left NAcc had greater frequency power in the MF band (p=.009) (**Figure 2A**). There was no significant correlation between the left NAcc brain volume and frequency power in either the MF (r=.228, p=.115) or LF (r=.126, p=.388) bands. The bilateral ACC (left p<.001, right p=.014) and vmPFC/OFC (left p<.001, right p=.034) showed greater frequency power in the LF band (**Figure 2B**). There were no brain regions that had greater frequency power in the lean subjects. None of the other ROIs showed significant group differences.

Functional connectivity of the NAcc

The region within the left NAcc and the other regions (bilateral ACC and vmPFC) identified in the above frequency analysis were used to determine the functional connectivity between these regions. These results are summarized in **Table 3**. The left NAcc region demonstrated band-specific altered functional connectivity with the ACC and vmPFC in the high BMI group compared to the lean group. In the MF band, greater NAcc functional connectivity was observed in the overweight and obese group with left and right ACC (p=0.024; p=0.024), and right vmPFC/OFC (p=0.0315) (**Figure 3A**). In the LF band, greater NAcc functional connectivity was observed with the left ACC (p=0.03) (**Figure 3B**). Functional connectivity and BMI was found to be significantly correlated for all the regions that had greater functional connectivity with the defined cluster of the left NAcc in the high BMI group for both MF [right ACC (p<.001, r=.522), left ACC (p=.013, r=.349), and right vmPFC/OFC (p<.001, r=.517)] and LF [left ACC (p=.023, r=.321)] bands. No NAcc functional connectivity was observed to be greater in the lean group.

Discussion

In this study, we examined differences between healthy women with high and normal BMI regarding NAcc volume, frequency distribution of intrinsic BOLD oscillations, and in terms of NAcc functional connectivity. The main findings of the study were: Women with increased BMI had 1) significantly larger left NAcc volumes, 2) increased band-specific frequency power in key regions of the extended reward network, including the NAcc, and 3) increased functional connectivity of a region within the NAcc with other extended reward-related regions that had also demonstrated altered frequency. The findings of this study are consistent with an alteration in central reward circuits in the pathophysiology of obese women. To our knowledge, this is the first study to demonstrate abnormalities in the intrinsic oscillation and connectivity of the RS brain in women with increased BMI.

Increased BMI is associated with larger NAcc volume

Our findings of increased GMV of the NAcc are consistent with previous reports that focused on male/female differences (20) and adolescent (24) and elderly (46) subjects showing greater GMV in reward regions with higher BMI. Based on positive correlations between serum leptin levels and greater GMV in NAcc and ventral striatum, it has previously been suggested that the obesity related structural differences may be a consequence of increased signaling from adipose tissue to the brain,[12] rather than a consequence of altered ingestive behavior. Further support for a hypothesized role of adipocyte related signals influencing brain structure comes from a recent study in morbidly obese subjects, in which structural brain changes were negatively correlated with body fat.[36] Brain imaging findings from other disorders of ingestive behavior (e.g. anorexia nervosa, bulimia nervosa) also suggest the possible role of signals related to body fat or hydration, rather than to food intake or addiction in the development of structural brain differences. For example, a systematic review done by Van den Eyden[37] suggests there is a general increase in volume of brain regions in bulimia nervosa and binge eating disorder which are ingestive behavioral disorders that are associated with increased eating. However, there is a general decrease in brain volumes observed in anorexia nervosa, a disorder characterized by severe reduction in food intake and loss of body fat.[37] Together these studies suggest that adipocyte related signaling to the brain may result in both increases as well as decreases in GMV and that these alterations are based on the degree of food and water intake. Mechanistic studies in rodent models are required to further test this hypothesis.

Increased spontaneous brain oscillations in individuals with high BMI within the low and medium frequency bands

In order to identify specific regions for the functional connectivity analyses, we first performed a regional frequency analysis of spontaneous resting state oscillations. Frequency power differences showed that women with increased BMI had a greater mean amplitude in the LF and MF bands relative to the entire frequency spectrum in left NAcc and bilateral ACC and vmPFC, (i.e. regions of the extended reward network). The fact that no significant correlations were found between the left NAcc volume and oscillatory amplitude in the MF or LF bands suggests that there is no simple relationship between structural and resting state differences.[38-40]

While the functional consequences of differences in regional brain oscillations are not fully understood,[31, 41] we may speculate about possible interpretations relevant to ingestive behavior and obesity. One interpretation of increased frequency power in lower bands (including both LF and MF bands) is that it reflects reduced neuronal firing.[41, 42] Reduced activity in the NAcc could reflect the loss of Dopamine 2 Receptors (D2Rs) in this brain region which has previously been reported in obese subjects, and is associated with impaired DA signaling[43] and a decrease in the inhibitory role of the NAcc.[44] Reduced activity in the ACC and vmPFC could also reflect altered DA signaling as decreased availability of striatal D2Rs has previously been linked to decreased activity in the PFC and ACC in both obesity and drug addiction.[45, 46] These cortical regions are involved in inhibitory control.[47] It has been hypothesized that reduced engagement of the PFC/ACC contributes to the lack of behavioral control in individuals with different forms of addiction.[6]

The separation of frequency bands used in this analysis was based on the observation that behaviorally relevant brain oscillations have linearly distributed center frequencies on the natural logarithmic scale.[25] Even though the functional relevance of differences obtained with this

analysis method are incompletely understood, [41, 42, 48, 49], it has been used in several published reports.[15, 30, 38-40, 50, 51] For example, variability in frequency power has been studied in mild cognitive impairment[50] as well as Parkinson's where treatment-related changes in frequency power of motor cortex/basal ganglia have been related to motor performance.[15, 51] Kilpatrick et. al. identified band-specific BOLD fluctuation differences after ingestion of high-sucrose compared with low-sucrose beverages in the nucleus tractus solitarius which also demonstrated altered band specific functional connectivity.[30] These studies provide support that band-specific alterations in frequency power are functionally relevant and can be used to identify affected brain regions with altered activity/connectivity.[30]

Increased NAcc functional connectivity with cortical reward-related regions in subjects with high BMI

The increased functional connectivity between the left NAcc cluster with the ACC and vmPFC in the high BMI group compared to the lean group in this study is similar to findings reported in individuals addicted to nicotine and other drugs.[52, 53] In heroin users,[54] the observed increased functional connectivity between regions involved in reward, craving, and motivation (e.g. between NAcc, ACC, and OFC) were thought to be related to compulsive behaviors characteristic of heroin addicts. An increase in functional connectivity between the NAcc and cortical control regions has also been observed in other compulsive disorders, not related to substance abuse.[53, 55] For example, in Obsessive Compulsive Disorder patients, greater functional connectivity of the ACC with regions including the NAcc is suggested to contribute to the loss of control observed in these subjects.[55]

Limitations

The unilateral structural and functional alterations observed for the (left) NAcc may be a consequence of sample size (as a trend was observed for the right NAcc), or it may reflect a true laterality. Future studies in larger populations need to address this question. There is limited information on the interpretation of frequency band oscillations and their physiological significance. Given that activation of brain regions through a task has been shown to shift frequency distribution towards higher frequencies,[56] one possible interpretation of the increased lower frequency power is that these regions are less active; however, other interpretations are possible.[41, 42] For example, band-specific alterations in frequency power may reflect altered balance between local processing versus long-range inputs with increased MF power reflecting greater local processing. It has also been suggested that examining functional connectivity of various bands would have similar physiological implications.[31]

No autonomic measures were collected to address how they might impact RS measures.[57, 58] However, we controlled for possible influences of autonomic activity on resting state oscillations by regressing out nuisance variables and focusing on bands less influenced by physiological noise (LF, MF). Since the subjects used in the study were collected from a database, we did not have consistent information in regards to dietary habits, fasting, appetite, and hunger. As a result, we were not able to correlate the observed biological abnormalities with behavioral patterns of food intake. Also, due to the nature of the data collection, time of day for the scans is variable. Finally, the study was performed only in female subjects. Several studies have reported sex-related differences in obesity, addiction and related neurobiology[12, 59, 60] and it is therefore likely that findings may differ in a male population.

Summary and Clinical Implications

Our results demonstrating structural, functional and connectivity alterations in women with increased BMI support the important role of alterations within the extended reward network, including inhibitory cortical control mechanisms that can lead to the ingestive behaviors which are more driven by hedonic as opposed to metabolic aspects of food intake. As the subjects in this study were only selected by their differences in BMI and not by differences in behavioral measures of food intake (such as measures of food addiction), the findings suggest that such an increased influence of reward driven mechanism is likely to be present to varying degrees in obese subjects in general. However, the alteration may become the predominant mechanism in subjects with increasing scores on the food addiction questionnaire.[7] Future studies are needed to validate these observations in a larger group of male and female subjects with different degrees of behavioral food addiction, and to determine the potential use of these measures as a potential neurobiological biomarker for subsets of obese individuals. If confirmed, such biomarkers could be used to enrich patients for therapeutic trials, and to evaluate the effectiveness of therapeutic interventions.

Tables

Table 1. Subject Clinical Characteristics

	Lean N=31	**Overweight and Obese N=19**	**F**	**p-value**
Age (yr)	Mean: 25.42 Range: 18-38 SD: 5.86	Mean: 27.05 Range: 19-40 SD: 7.03	.940	.337
BMI (kg/m^2)	Mean: 22.32 Range: 19.52-25.09 SD: 1.85	Mean: 31.83 Range: 25.88-37.56 SD: 3.35	145.730	<.001
HAD Depression	Mean: .50 SD: 1.34	Mean: 1.64 SD: 2.09	.361	.552
HAD Anxiety	Mean: 2.17 SD: 2.84	Mean: 3.71 SD: 1.64	2.301	.139
NEO Neuroticism	Mean: 40.96 SD: 11.26	Mean: 45.64 SD: 10.49	.268	.610
ETI:				
Total	3.08 (3.11)	5.50 (4.53)	1.010	.324
General	1.44 (1.16)	2.07 (1.83)	.701	.410
Physical	.78 (1.22)	1.40 (1.77)	.805	.378
Emotional	.37 (1.12)	1.40 (1.77)	.651	.427
Sexual	.41 (1.05)	.53 (.74)	.003	.957

BMI: Body Mass Index;

HAD: Hospital Anxiety and Depression;

ETI: Early Traumatic Inventory

P values significant: .05*, .01**

Table 2: Differences in Nucleus Accumbens Volumes Between Obese/Overweight and Lean Females

	Lean Women	Overweight and Obese Women	F-value (Left NAcc vs. Right NAcc)	p-value
Left NAcc	569.76	636.25	4.963	.031*
Right NAcc	479.11	532.96	2.989	.091

NAcc: nucleus accumbens

p-values significant: .05*, .01**

Table 3: fALFF Analysis for Medium Frequency (MF) and Low Frequency (LF) Bands

	Coordinate (x,y,z)	**Cluster value (k)**	**p-value (FWE)***	**Z-score**
Greater MF (0.027-0.073 Hz) power in Obese/Overweight compared to Lean				
L NAcc	(-14, 12, -12)	21	0.009	4.29
Greater LF (0.01-0.027 Hz) power in Obese/Overweight compared to Lean				
L ACC	(-6, 52, -6)	212	<.001	6.00
R ACC	(0, 52, -4)	62	0.014	4.71
L vmPFC	(-6, 54, -18)	130	<.001	5.14
R vmPFC	(4, 48, -14)	35	0.034	4.00

Comparing groups in a fractional amplitude of low-frequency fluctuation (fALFF) band analysis, greater frequency power was observed in certain brain ROIs associated with reward in the Medium MF (0.027-0.073 Hz) and Low LF (0.01-0.027 Hz) bands for the overweight and obese group. The results for the following regions were not significant: hippocampus, orbital frontal cortex, amygdala, insula, caudate, putamen, pallidum. No regions were found to have greater frequency power in the lean group. *p value listed were observed after correction for multiple comparisons NAcc= Nucleus Accumbens, ACC = Anterior Cingulate Cortex, vmPFC = Ventromedial Prefrontal Cortex, L = left, R = right.

Table 4: Nucleus Accumbens (NAcc) Functional Connectivity in Overweight and Obese > Lean

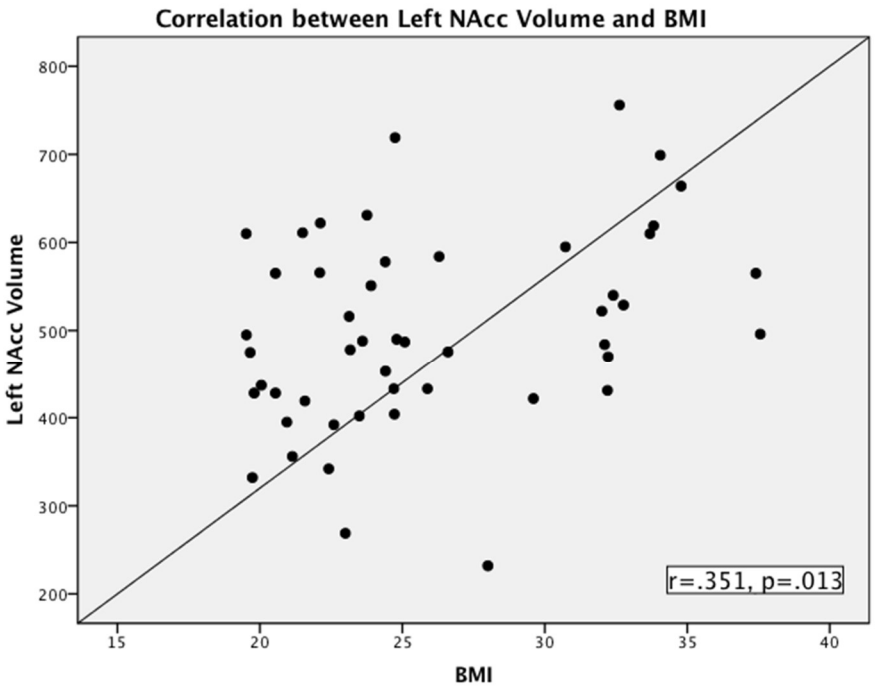
	Coordinate (x,y,z)	**Cluster value (K)**	**p-value (FWE)***	**Z-score**
Greater NAcc MF functional connectivity (0.027-0.073 Hz) in Obese/Overweight compared to Lean				

L ACC	(-2 42 12)	43	0.024	3.71
R ACC	(8 34 -14)	53	0.024	4.08
L vmPFC	(-14 16 -16)	2	0.154	4.34
R vmPFC	(6 34 -16)	28	0.0315	4.04
Greater NAcc LF functional connectivity (0.01-0.027 Hz) in Obese/Overweight compared to Lean				
L ACC	(-14 38 -12)	49	0.03	3.78
R ACC	(12 46 -12)	13	0.063	3.43
L vmPFC	(-2 34 -16)	24	0.051	3.49
R vmPFC	(10 34 -20)	17	0.051	3.82

Using the nucleus accumbens (NAcc) as a seed, greater functional connectivity was observed in the overweight and obese compared to the lean in both the MF (0.027-0.073 Hz) and LF (0.01-0.027 Hz) bands for the tested ROIs (bilateral ACC and VMPFC). ROIs chosen based upon significant results of the fALFF analysis (Table 3). No regions were found to have greater functional connectivity in the lean group. *p value observed after correction for multiple comparisons. NAcc= Nucleus Accumbens, ACC = Anterior Cingulate Cortex, vmPFC = Ventromedial Prefrontal Cortex, L = left, R = right.

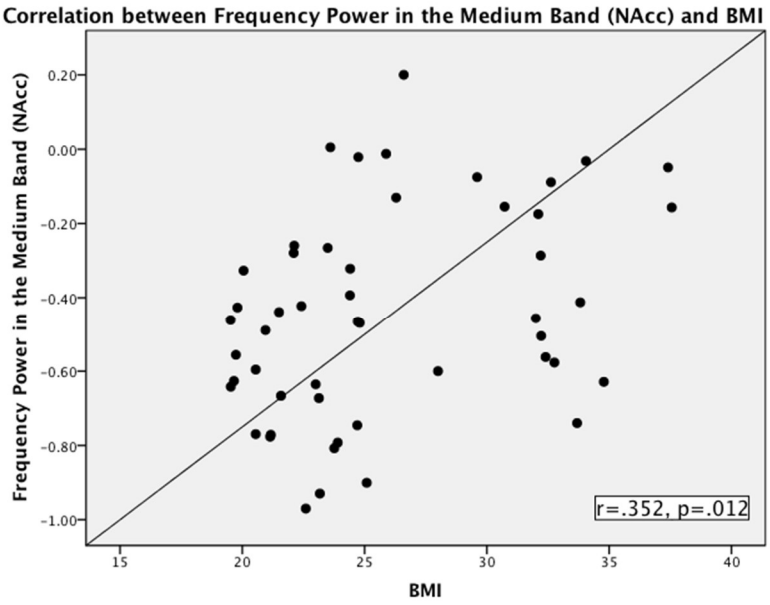
Figures

Figure 1. Correlation Between Left Nucleus Accumbens (NAcc) and Body Mass Index (BMI)



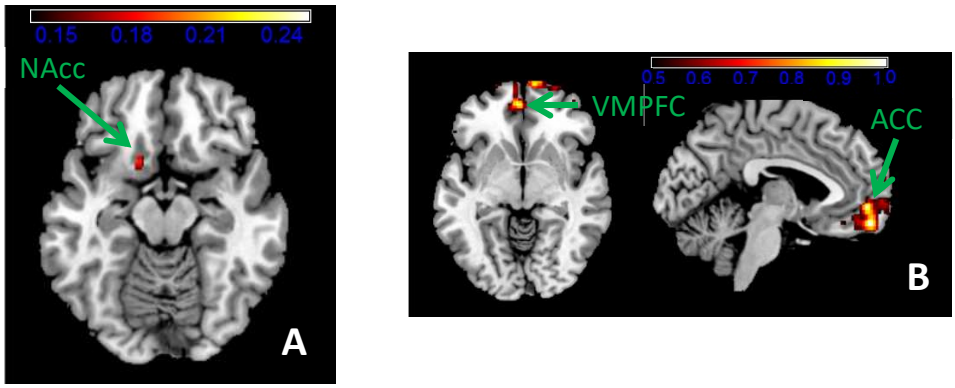
Significant positive correlations were seen for the left nucleus accumbens (NAcc) volume with increasing body mass index (BMI) (r=.351, p=0.013).

Figure 2. Correlation Between Frequency Power in the Medium Band (MF) for the Left Nucleus Accumbens (NAcc) and Body Mass Index (BMI)



Significant positive correlations were seen for the frequency power in the medium band (0.027-0.073 Hz) for the left nucleus accumbens (NAcc) with increasing body mass index (BMI) (r=.352, p=.012).

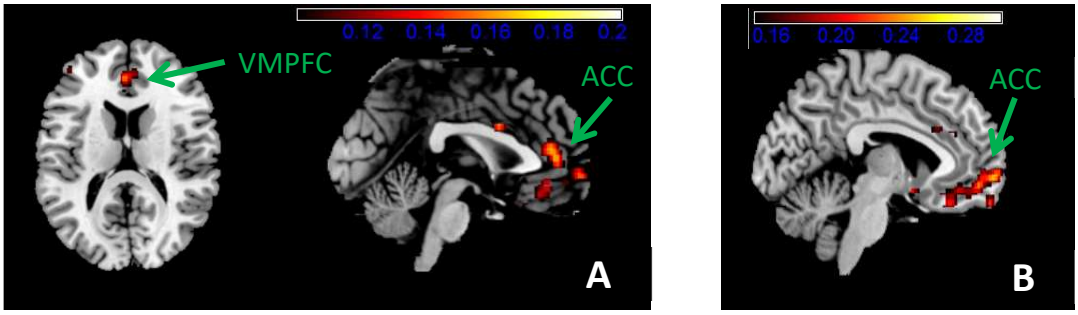
Figure 3: Fractional amplitude of low-frequency fluctuation (fALFF) analysis in the overweight and obese group compared to the lean group for both A) Medium (MF: 0.027-0.073 Hz) and B) Low (LF: 0.01-0.027 Hz) frequency bands



In the MF band (A), greater frequency power was observed in the left nucleus accumbens (NAcc).

In the LF band (B), greater frequency power was observed in the anterior cingulate cortex (ACC) and ventromedial prefrontal cortex (vmPFC).

Figure 4. Functional Connectivity analysis of left nucleus accumbens (NAcc) with other reward-based regions in the overweight and obese group compared to lean



Functional Connectivity analysis using the left nucleus accumbens (NAcc) as a seed region showed greater functional connectivity in the overweight and obese group (compared to lean) with reward-based regions of altered frequency identified in the fALFF analysis.

In the MF band (A), greater connectivity observed between the seed region and the ACC, and R VMPFC.

In the LF band (B), greater functional connectivity was observed between the seed region and L ACC.

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CHAPTER FIVE

Background: The Impact of Satiety Hormones on the Brain

The Role of the Gut Brain Axis in Ingestive Behavior

Overview

The impact of gut peptides on the brain in the regulation of ingestive behavior is an area of great interest, since the involved signaling mechanisms may be potential targets for the treatment of obesity. Both endocrine, paracrine and neurocrine (vagal) signaling pathways are involved in gut to brain signaling of nutrient related information(1). Human studies have demonstrated that long acting hormones like leptin and insulin act on brain regions such as the hypothalamus and brainstem, influencing both food intake and response(2). More short-term gut signals relayed by hormones such as CCK and PYY have also been shown in humans to have an effect on subcortical regions, including the hypothalamus, NTS, and thalamus), as well as cortical regions like the insula and anterior cingulate cortex(2). Whereas CCK infusion in healthy subjects resulted in increased activity primarily in homeostatic brain regions (hypothalamus, brainstem), PYY infusions were associated with increased activity in regions of reward circuits (orbitalfrontal cortex, insula, anterior cingulate cortex). The central effects of GLP-1 in humans have not been as thoroughly studied(2).

GLP-1/Exenatide and The Brain

GLP-1

Glucagon like Peptide-1 (GLP-1) is a gut hormone synthesized in ileal L cells of the small intestine and released postprandially in response to carbohydrates and fat(3). Like other gut peptides, GLP-1 exerts its actions via endocrine and via paracrine/vagal signaling pathways. Its primary function is to stimulate insulin secretion from pancreatic beta cells and the peptide is therefore referred to as an incretin. GLP-1 is also produced in the vagal nucleus of the solitary track(4), a brainstem region which plays a central role in the regulation of satiety.

GLP-1 has multiple actions including the suppression of glucagon release, the slowing of gastric emptying, and the increase of glucose utilization in the liver. GLP-1's influence on glucose homeostasis has made it a prime candidate for regulating glucose metabolism in patients with diabetes(5). Studies of GLP-1 in diabetes have reported the hormone to be linked to a decrease in food intake and an increase expression of satiety(6, 7, 8). Surgery induced increases in postprandial GLP-1 levels have been implicated in the observed weight loss following bariatric surgery(3). However, the mechanism(s) by which GLP-1 exerts its inhibitory effect on food intake and resulting weight loss are not fully known.

Exenatide

Due to its short half-life, synthetic GLP-1 receptor agonists such as Exenatide are used to provide long term GLP-1 receptor stimulation(9). Exenatide is a synthetic variant of exendin-4, a hormone extracted from the saliva of the Gila monster that bears 50% amino acid homology to GLP-1. Exenatide is FDA approved for the long term treatment of diabetes. Studies looking at the impact of Exenatide have reported that similar to GLP-1, the drug reduces food consumption and leads to weight loss, even though the effect of the drug on feelings of satiety or hunger have been inconsistent(3, 8).

The impact of GLP-1 when it comes to obesity and weight loss is becoming clearer, but the role of the brain and how GLP-1 might impact its structure and function is less studied. The production of GLP-1 in the nucleus of the solitary tract suggests the hormone has an impact on the brain. The nucleus of the solitary tract sends projections to the hypothalamus and to interoceptive regions within the brain's homeostatic network(10, 11). Vagal signaling to the hypothalamus and thalamus is also likely to play a crucial role in mediating the impact of GLP-1 on ingestive behavior(12). Alterations of this signaling has been implicated in the

pathophysiology of obesity(13) and this study aims to help define what those drug-induced alterations might be and how they would differ between health lean and obese women.

Exenatide Pharmacokinetics

Exenatide pharmacokinetics have been studied in patients with type 2 diabetes. In this population, subcutaneous administration of 10-mcg of Exenatide reached median peak plasma concentration in 2.1 hours. The mean peak Exenatide concentration (C_{max}) was 211 pg/ML and overall mean area under the time-concentration curve ($AUC_{0\text{-}inf}$) was 1036 pg*h/mL. Exenatide exposure increased proportionally over the therapeutic dose range of 5 mcg to 10mcg. The C_{max} values increased less proportionally over the same range. Gender and race do not influence the distribution and elimination of Exenatide. Similar exposure is achieved with subcutaneous administration of BYETTA in the abdomen, thigh, or upper arm. In most individuals, Exenatide concentrations are measurable for approximately 10 hours post-dose (14).

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CHAPTER SIX

Manuscript: The Effect of the GLP-1 Analogue Exenatide on Functional Connectivity within an NTS-Based Network in Lean and Obese Females

Abstract

Objective: To investigate the differential effect of the GLP-1 agonist Exenatide on the functional connectivity of brain networks and behavioral data in healthy lean and obese females.

Methods: Following an 8 hour fast, 19 healthy female subjects (11 lean, 8 obese) participated in a two-day double blind crossover study. Subjects underwent functional magnetic resonance imaging (fMRI) at fast and 30 minutes after a subcutaneous injection of 5ugs Exenatide or placebo. Functional connectivity was examined using seeds for the Nucleus Tractus Solitaries (NTS). Drug-induced functional connectivity changes within and between groups and correlations between functional connectivity and appetite measures were examined in a region of interest approach focusing on the thalamus and hypothalamus. **Results**: When compared to the lean group, the obese subjects reported less hunger after drug injection. Exenatide administration increased functional connectivity of the left NTS with the left thalamus and hypothalamus in the obese group only. Exenatide increased the positive correlation between NTS functional connectivity and hunger scores in all subjects, but more so in the obese. **Conclusions**: 1. Obesity can impact the effects of Exenatide on brain connectivity. 2. The drug's impact on appetite control might be linked to modified connectivity in an NTS-based network.

Introduction

Glucagon like Peptide 1 (GLP-1) receptor agonists have shown to improve glucose control, decrease food intake, accelerate weight loss and increase satiety (1, 2, 3). Increases in postprandial GLP-1 levels have also been implicated in the observed weight loss following bariatric surgery (1, 4). Because of these effects, the role of GLP-1 in the regulation of food intake has been evaluated (5).

GLP-1 is primarily released from L cells in the distal gut, but it is also produced in the vagal nucleus of the solitary tract (NTS) (6), a brainstem region which plays a central role in the regulation of satiety. The NTS sends projections to the hypothalamus and interoceptive regions within the brain's homeostatic network (including thalamus and insular cortex) (7, 8). Vagal signaling to the hypothalamus and thalamus is likely to play a crucial role in mediating the impact of GLP-1 on ingestive behavior (11) and abnormalities in this signaling have been implicated in the pathophysiology of obesity (9). While findings from rodent studies emphasize the important interactions between GLP-1 and NTS on body weight control and human studies have shown reduced appetite in human subjects in response to Liraglutide and other GLP-1 agonists, the central mechanisms of this molecule are incompletely understood (10, 11).

Functional brain imaging techniques help bridge the gap between clinical observation and underlying neurobiological mechanisms. Measuring intrinsic brain oscillations during resting conditions has demonstrated altered functional connectivity of specific brain regions in clinical conditions including obesity (12). We have previously demonstrated altered NTS functional connectivity with the hypothalamus between lean and obese women while looking at images of palatable food after consuming a caloric beverage (13). These brain changes were correlated with alterations in taste ratings determined by a questionnaire, linking functional connectivity abnormalities in the NTS and hypothalamus to ingestive behavior (13).

Based on our previous observations of differential functional connectivity in obese and lean subjects, the current study aimed to measure the impact of the GLP-1 analog Exenatide on the intrinsic functional connectivity of the NTS with other brain regions. By studying resting state activity in healthy lean and obese female subjects, we aimed to test the following hypotheses: 1) Exenatide changes functional connectivity between NTS and the thalamus and hypothalamus, 2) These drug-induced changes in functional connectivity are different in lean and obese females, and 3) The alterations in NTS functional connectivity are correlated with behavioral measures related to eating such as calories consumed at a meal and subjective feelings of hunger.

Methods

Subject Selection

Nineteen healthy female subjects, ages 18-40, were recruited through the Oppenheimer Family Center for Neurobiology of Stress from flyers and website advertisements. The sample included 11 lean females (mean age: 25.09± 4.83 years old) with a Body Mass Index (BMI) range of 19.52-24.74 kg/m^2 and 8 obese females (mean age: 26.62±7.63 years old) with a BMI range of 30.72-37.56 kg/m^2. All subjects were right-handed, pre-menopausal and in the follicular phase of the menstrual cycle by self-report, and classified as healthy after a clinical assessment that included a modified Mini-International Neuropsychiatric Interview Plus 5.0(14), a brief structured interview for major Axis I psychiatric disorders in DSM-IV(15) and ICD-10 (16). Further exclusion criteria included pregnancy, substance abuse, tobacco dependence, evidence of cardiovascular, gastrointestinal, hepatic, neurologic, or psychiatric illness, diabetes, high blood glucose levels (<200mg/dl), strenuous exercise (more than 8 hours/week), and any eating disorders. Subjects were also excluded if they were currently on medication such as analgesics or antidepressants, had used any diet aids in the last month, had undergone any obesity-reduction surgeries, were not able to undergo an MRI, or could not eat one of three meal options. Throughout the study, subjects were asked to report any feelings of nausea. The subjects provided written informed consent and all procedures were reviewed and approved by the UCLA Medical Institutional Review Board.

Study Paradigm

Subjects came in for three visits, a screening visit to determine their eligibility followed by two nearly identical MRI visits where they received either the drug or saline placebo injection in a randomized, double-blinded fashion. All scanning began in the morning after at least eight hours of fasting (subjects did not eat breakfast). Blood glucose levels were determined to be within the range of 65-126mg/dl. Brain scans were obtained at a pre-injection baseline and at 25 min after drug/placebo administration. Blood samples were obtained at fasting, before the drug/placebo injection and at 25, 35 and 60 min after drug administration. Scores for hunger/satiety levels using a 100-point visual analogue scale were obtained at the same time as the blood draws. After scanning, subjects were taken to a general clinical research area and given a pre-selected 1000 calorie meal composed of 30% fat, 20% protein, and 50% carbohydrates and were instructed to “eat until you are full”. Meals were measured before and after consumption to determine calories consumed.

The basic protocol is reflected in Figure 1.

MRI protocol

Before placement into the scanner, subjects completed the Patient-Reported Outcome Measurement Information Systems (PROMIS) (17) and the Hospital Anxiety and Depression questionnaire (18). Following placement in the scanner, subjects underwent a 3 minute alignment true fast imaging with steady-state free precession (TRUFI) scan before a baseline 10 minute resting state scan during which they were asked to lie still with their eyes closed and instructed not to fall asleep. After the resting scan, subjects were removed from the scanner to receive either a subcutaneous injection of 5ug of Exenatide or a 0.5cc injection of saline (placebo) in the deltoid (upper arm) area. After the injection, subjects were moved back into the

scanner for a 9 minute standard T1-weighted magnetization-prepared rapid acquisition gradient echo (MP-RAGE) structural scan followed by a 10-min resting state scan that was completed 25-30 minutes after the injection.

Resting State Data Acquisition and Preprocessing

Functional MRI resting state scans were acquired on a Siemens 3 Tesla Trio scanner. The ten minute scans were acquired with an echo planar sequence with the following parameters: TE = 28ms, TR = 2000ms, flip angel = 77 degrees, FOV = 220mm, slices = 40, slice thickness = 4.0mm. Slices were obtained with whole-brain coverage.

Image processing and data analysis were performed using Statistical Parametric Mapping 8 (SPM8) software (Wellcome Department of Cognitive Neurology, London). Processing was done through the SPM toolbox, Data Processing Assistant for Resting-State fMRI (DPARSF) (19). Data was slice-time, motion corrected, and nuisance covariate regression was preformed to minimize physiological noise using six head motion parameters, white matter signal and cerebrospinal fluid signal. Data were spatially normalized to the Montreal Neurological Institute (MNI) template using the MP-RAGE structural scan which was acquired with the following parameters: TE = 3.26 ms, TR = 2200ms, slices = 176, slice thickness = 1.0mm, voxel size = 1x1x1mm. Spatial smoothing with a $4mm^3$ Gaussian kernel occurred after calculation of connectivity maps (19).

Blood Sample Processing

Blood samples were sent to outside laboratories for processing. Tamdem Labs California used an enzyme-linked immunosorbent assay (ELISA) to determine plasma concentration of Exenatide (20, 21).

Data Analyses

Clinical/Behavioral Characteristics

Analyses of clinical characteristics (age, Body Mass Index (BMI), education, anxiety, depression), behavioral measures (calorie consumption, fullness questionnaires), and blood levels of Exenatide were performed in SPSS 22 (22). Clinical characteristics were evaluated using t-tests to compare group means (lean; obese). Anxiety and depression scores were compared separately for each day (screening day, drug day, placebo day). Levels of Exenatide were compared between each group (lean; obese) by both day (drug; placebo) and time post-injection (35 min; 60 min). In order to determine calorie consumption, a GLM was run looking at group (lean: obese) x day (drug; placebo) using blood levels of Exenatide as a covariate. Behavioral measures from the fullness questionnaire were analyzed with ANOVAs looking at group (lean; obese) x time (baseline; 35min post-injection, 60min post-injection) and changes over time (i.e. 60 min post-injection > 35 min post-injection) for changes between days (drug day – placebo day).

Seed-based Functional Connectivity of the NTS

Using MarsBar (23), an NTS seed cluster was defined by a 4mm sphere around the peak NTS coordinate determined by Kilpatrick et al (13). Left and right clusters were defined separately. The functionally defined NTS cluster is simply referred to as the NTS in results and discussion

sections. Fisher transformed maps of the bivariate correlation between the NTS seed time-course and all other voxels were created using DPARSF (19) for the left and right NTS. Based on significant results from Kilpatrick et al, this was done for a specific low frequency band (0.01-0.027 Hz) (13, 24). The resulting functional connectivity maps were analyzed in a flexible factorial General Linear Model (GLM) in SPM8 to compare group data (obese; lean) x day (drug; placebo) x scan (pre-injection; post-injection). The order of the drug day was included as a factor in the model to account for any order effects. Age and blood levels of Exenatide were used as covariates. Using a region of interest (ROI) approach similar to other studies (12, 25), contrasts were performed to identify regions with altered connectivity with the NTS before or after the drug versus placebo, looking at both within and between group differences. Anatomically-based ROIs were created using the Wake Forest University PickAtlas toolbox in SPM8. Hypothalamus and thalamus ROIs were chosen based on previous literature defining them as regions associated with the NTS in hunger and satiety signaling (9). Images were thresholded at p=.001, uncorrected and small volume corrections was applied to determine significance at p<.05, corrected for family wise error (FWE) rate. False discovery rate (FDR) was applied to control for the type I error inherent in testing multiple ROIs (26, 27). Cluster size was limited to Ke>3.

Functional Connectivity Correlations with Behavior

A multiple regression model in SPM8 was used to determine group differences in the association of calorie consumption and fullness questionnaire scores with functional connectivity of the NTS seed regions and designated ROIs described above. Blood levels of Exenatide, age, and order of the drug day were used as covariates. The questionnaire responses selected for the correlational analysis were those that showed significant group differences in the behavioral SPSS analyses, which related to hunger. Contrast images were created to identify drug-induced changes in the correlation between functional connectivity and behavioral measure

both within and between groups, as well as differences at the pre-injection baseline. Images were thresholded at p=.001, uncorrected and small volume correction was employed to determine significance of the ROI at p<.05 FWE with additional FDR correction to account for multiple comparisons (26, 27). Cluster size was limited to Ke>3.

Results

Clinical Characteristics

There were no statistically significant group differences in age or any clinical variable other than BMI (Table 1). With all subjects in the clinically normal range, the two groups did not differ on any visit in anxiety symptom scores (screening: F=3.46, p=.080; placebo day: F=1.233, p=.282; drug day: F=3.28, p=.088) or depression symptom scores (screening: F=4.139, p=.058; placebo day: F=.370, p=.552; drug day: F=.306, p=.558) (Table 1).

Exenatide Levels

Both groups showed significantly higher levels of Exenatide in blood on the drug day compared to placebo day at all time points (p<.001) (Supplemental Table 1, Supplemental Figure 1). During the drug day, the lean group had higher plasma levels of Exenatide than the obese group both at 35 minutes (161pg/mL vs 97.5pg/mL), lean vs obese (p=.004) and 60 minutes (238.3pg/mL vs 147.5pg/mL, lean vs obese p=.001) after injection, but both groups showed an increase of the drug between those times (p=.007). Due to the pharmacokinetic value differences between the groups, all subsequent analyses used the level of Exenatide in the blood as a covariate.

Glucose Levels

All subjects combined on the drug day showed a decrease in mean blood glucose levels at both 35 minutes (77.35 mg/dl vs 86.56 mg/dl) and 60 minutes (75.87 mg/dl vs 86.56 mg/dl) after Exenatide injection compared to the placebo day ($p<.001$). Both lean and obese groups showed a significant reduction in mean blood glucose levels on the drug day between baseline and both 35 and 60 minutes post injection [lean baseline (83.8mg/dl) vs 35 minutes (75.90 mg/dl), $p= 0.026$; lean baseline vs 60 minutes (73.78 mg/dl), $p= 0.030$; obese baseline (90.57mg/dl) vs 35 minutes (79.43mg/dl), $p=0.007$; obese baseline vs 60 minutes (78.57mg/dl), $p=0.004$]. There were no significant differences between lean and obese groups (Supplemental Figure 2).

Hunger and Appetite Measures

There were no statistically significant group differences in calorie consumption on drug (468.21kcal vs 620.25kcal $p=.109$, $F=1.27$) or placebo (569.10kcal vs 652.81kcal $p=.275$, $F=2.86$) days. However, trends suggest both groups consumed less calories on the drug day and that the obese group consumed more calories on both days than the lean group (Figure 2).

Regarding hunger scores, the only significant change was seen in the obese group on the day of the Exenatide injection (versus the placebo injection), when they reported to be less hungry than the lean group 35 minutes after drug injection (8.63 vs -8.75; $p=.035$) and have a greater decrease in hunger scores after eating the provided meal (7.27 vs -10.00; $p=.025$). No significant group differences in hunger were observed before injection (Table 2). No group differences were observed for satiety levels.

Functional Connectivity of the NTS

No differences in functional connectivity were observed at baseline between day or group. After Exenatide administration, in comparison to the placebo, the obese group demonstrated increased functional connectivity of the left NTS with the left thalamus and left hypothalamus. No drug-induced connectivity change was observed in the lean group. For the right NTS seed, the obese group, compared to the lean group, showed greater functional connectivity with the left thalamus after drug administration versus the placebo (Table 3, Figure 3).

NTS Functional Connectivity and Behavioral Correlates

Calorie Consumption. For all subjects combined, there was a significant increase in the correlation between calorie consumption and functional connectivity of the right NTS with the right hypothalamus following Exenatide administration (versus placebo), (Table 4A, and Figure 4A). No group differences of significant correlations were observed before or after the drug injection. No subject consumed the entire meal.

Hunger Ratings. At baseline, lean subjects showed a statistically greater correlation between hunger scores and functional connectivity of the right NTS with the left thalamus [(-10 -16 16), p=.004, Ke=87, z=4.69] than obese subjects. After Exenatide injection (versus placebo), all subjects combined demonstrated a positive correlation between subjective feelings of hunger and functional connectivity of the right NTS with the right hypothalamus. Obese subjects had a statistically greater correlation between the hunger ratings and functional connectivity of the right NTS with the left thalamus compared to lean subjects and obese subjects only showed a correlation between hunger scores and functional connectivity of the left NTS with the left

thalamus (Table 4B, Figure 4B-D). No correlations were seen between NTS connectivity and satiety scores.

Discussion

The aim of this study was to identify brain and behavioral effects of the GLP-1 analog, Exenatide and evaluate how these effects differ between lean versus obese women. We show that Exenatide impacts both brain networks and behaviors related to hunger and satiety, generally with a greater impact in the obese.

Effect of Exenatide on Functional Connectivity of the NTS

Exenatide administration, in comparison to placebo, resulted in increased NTS functional connectivity with the thalamus and hypothalamus in the obese subjects. Specifically, an Exenatide-induced increase in NTS functional connectivity was seen in the obese only between the left NTS with the left hypothalamus and thalamus. For the right NTS, the functional connectivity with the left thalamus was statistically greater in the obese when compared to the lean group.

The brainstem, specifically the NTS, and the hypothalamus are regions associated with regulation of food intake and ingestive behavior (3). The bidirectional connection between these two regions is involved in integrating peripheral signals from hormones in the regulation of satiety (3) and differences in hypothalamus connectivity between lean and obese patients has been shown to be reduced after successful bariatric surgery (28). This increased connectivity in response to the drug suggests an Exenatide-induced alteration of satiety signaling in obese

individuals. Previous reports have suggested a decreased sensitivity to GLP-1 in obesity and have connected reduced endogenous GLP-1 and reduced GLP-1 receptor activation to weight gain (29). Imaging studies have linked increased receptor availability to increased functional connectivity in the brain. An exogenous GLP-1 agonist might be working to overcome a decreased sensitivity in the obese and the Exenatide-induced increase in functional connectivity we observed more so in the obese group could be reflective of the drug's ability to increase GLP-1 receptor activity in a depressed system.

This study also showed that after Exenatide injection, the obese group demonstrated significantly greater functional connectivity between the NTS and the thalamus compared to the lean group. Regions of the thalamus, particularly the paraventricular area, receive input from the NTS, and these inputs are thought to play a role in homeostatic regulation of food intake (30). A greater increase in functional connectivity with the thalamus in the obese could indicate that Exenatide may modulate homeostatic regulation of food intake differently in this group compared to more lean individuals.

Effect of Exenatide on Hunger and Appetite Measures

As previously reported, the behavioral results of this study confirmed that Exenatide impacts feeding behaviors showing changes in both calorie consumption and hunger levels in both lean and obese subjects (2, 3).

Despite the greater reduction in their hunger scores after Exenatide, obese subjects consumed more calories than lean subjects on both the Exenatide test day and placebo day. This finding could point to altered satiety signal processing, as suggested by several studies in animals and humans that have shown obesity-related resistance to satiety signals such as leptin, insulin, and GLP-1 at brain areas associated with eating control including the hypothalamus (29, 31, 32). It

is conceivable that the administration of Exenatide can overcome a baseline obesity-related resistance to GLP-1 satiety signals in obese women, whereas Exenatide in the lean women may have a more immediate and dramatic impact on calorie consumption. The observed difference in hunger levels in the obese subjects might also, in part, be attributed to other factors associated with adiposity and obesity (33).

Functional Connectivity Correlations with Behaviors Related to Appetite

When all subjects were combined, a stronger correlation between calorie consumption and functional connectivity of the right NTS with the left hypothalamus was observed after Exenatide injection. Exenatide has been shown to increase hypothalamic connectivity with the rest of the brain in obese non-diabetic male subjects while subjects were viewing pictures of food (34). Our study suggests an Exenatide-induced strengthening of the relationship between NTS connectivity with the hypothalamus and calorie consumption that can be observed independent of exposure to visual food cues.

All subjects combined also showed a stronger Exenatide-induced correlation between subject hunger ratings and functional connectivity between the right NTS and right hypothalamus. The NTS is a known relay for vagal information from the gut and projects to the hypothalamus, which in turn, communicates with emotion-regulating and higher cortical centers to generate sensations of hunger. Our results suggest the Exenatide-induced functional connectivity between these regions plays a role in subjective feelings of hunger, regardless of BMI.

At baseline, before Exenatide administration, we found a stronger correlation between subjective hunger and functional connectivity of the right NTS and left thalamus in the lean compared to the obese group. However, after Exenatide administration, the obese group demonstrated a stronger relationship between hunger and functional connectivity of the NTS

with the left thalamus, suggesting the drug's greater impact in strengthening the perhaps depressed engagement of an NTS-based network with feelings of hunger in the obese.

Limitations

The current results, obtained in a relatively small sample, will require validation in a larger sample. Several studies have reported sex-related brain differences in obesity and eating behavior, indicating that obese females have a greater brain response to food in regions associated with emotional processing and motivation (35). Larger studies would need to be performed to examine potential resting state differences between males and females.

There were also several measurements not collected that would have helped refine the results of this study. Resting state data may be influenced by factors beyond our measure, such as gastric emptying or other satiety hormones. Although we focused analyses on a frequency band less influenced by physiological noise, autonomic functions have been demonstrated to affect resting state information (36). Although we asked subjects to report any feelings of nausea and none did, nausea is a known side effect of GLP-1 agonists, and the NTS also plays a role in feelings of nausea, and could impact other brain changes in this region(37). Also, although the varying levels of blood Exenatide were considered for our analyses, future studies would benefit from taking a weighted dosage of Exenatide based on body fat composition or body weight. Future studies with more subjects would also benefit from more specific hypotheses and analyses for specific areas within a brain region known to be involved in the processing of food-related signaling from the gut.

Although BMI is the current clinically-used measure of obesity, it has been argued that other measures such as visceral fat or waist circumference may be better measures (38, 39). This study also did not take into account any potential individual variants in the causes of obesity. Subsequent studies might benefit from examining factors such as food addiction, genetics,

family history, stress, etc. to determine if there are any such measures that might differentiate the impact of Exenatide on brain connectivity results.

Summary and Clinical Implications

The release of several satiety hormones, including GLP-1, is stimulated by nutrient ingestion and modulates the response of the central nervous system to food intake. GLP-1 stimulation, both in the periphery and within the brain, can impact the interpretation of hunger and satiety signaling. An imbalance or misinterpretation of these signals is thought to play a role in obesity (9). While several studies examined the impact of GLP-1 analogs like Exenatide on stimulated brain responses, few have examined the impact of the drug on the resting brain. The current study demonstrates that following the acute administration of Exenatide, obese female subjects demonstrate a statistically significant decrease in the subjective feeling of hunger, and an associated increase in functional connectivity of the NTS, with the hypothalamus and thalamus, regions associated with eating behaviors. Exenatide also increased the positive correlation between the NTS-based functional connectivity and subjective feelings of hunger in all subjects, with a significantly greater impact in the obese group. As clinical trials are being conducted using GLP-1 analogs as a potential weight loss treatment (11, 40), the study of these hormones and their impact on the brain in obesity and obesity reduction is key to a complete understanding of the physiological mechanisms and potential effectiveness in the clinic.

Tables

Table 1. Subject Clinical Characteristics

	Lean N=11	**Obese N=8**	**F**	**p-value**
Age (yr)	Mean: 25.09 Range: 21-36 SD: 4.83	Mean: 26.62 Range: 19-39 SD: 7.63	-.538	.598

BMI (kg/m2)	Mean: 21.38 Range: 19.52-24.74 SD: 1.92	Mean: 34.08 Range: 30.72-37.56 SD: 2.35	168.54	<.001**
Education	Mean: 4.55 SD: 2.34	Mean: 4.00 SD: 1.78	.305	.588
HAD Anxiety				
Screening	Mean: 2.91 SD: 3.21	Mean: 5.50 SD: 2.67	3.46	.080
Placebo Day	Mean: 3.00 SD: 4.05	Mean: 4.75 SD: 2.12	1.233	.282
Drug Day	Mean: 2.54 SD: 3.59	Mean: 5.00 SD: 1.51	3.28	.088
HAD Depression				
Screening	Mean: .727 SD: 1.01	Mean: 1.87 SD: 1.46	4.139	.058
Placebo Day	Mean: 1.09 SD: 2.21	Mean: 1.63 SD:1.30	.370	.552
Drug Day	Mean:1.18 SD: 2.56	Mean: 1.75 SD: 1.58	.306	.588

BMI: Body Mass Index

HAD: Hospital Anxiety and Depression

P values significant: .05*, .01**

Education scoring: 1=8th grade or less; 2=Some high school; 3=High school graduate; 4=Some college; 5=College graduate; 6=Any post-graduate work

Table 2. Fullness Questionnaire Response

Q: How Hungry Do You Feel?			
Group	**Time Point**	**Mean**	**SD**
Lean	Baseline	0	29.07
	+35 minutes	8.63	16.14
	+ 60 minutes	2.73	16.33
	+35 > Baseline	8.64	31.71
	+60 > Baseline	2.73	37.77
	+60 > +35 minutes	7.27	18.08
Obese	Baseline	-8.75	36.03

	+35 minutes	-5.00	5.34
	+ 60 minutes	-2.50	3.78
	+35 > Baseline	3.75	38.89
	+60 > Baseline	6.25	34.10
	+60 > +35 minutes	-10.00	9.64
Group Difference	**Time Point**	**F**	**p-value**
Lean vs Obese	Baseline	.344	.565
	+35 minutes	5.221	.035
	+ 60 minutes	.777	.390
	+35 > Baseline	.091	.766
	+60 > Baseline	.044	.837
	+60 > +35 minutes	6.00	.025
Q: How Full Do You Feel?			
Group	**Time Point**	**Mean**	**SD**
Lean	Baseline	5.45	13.62
	+35 minutes	-4.55	16.35
	+ 60 minutes	-3.64	13.80
	+35 > Baseline	-10.00	19.34
	+60 > Baseline	-9.09	18.00
	+60 > +35 minutes	-1.36	26.00
Obese	Baseline	-.625	21.78
	+35 minutes	7.50	10.35
	+ 60 minutes	-3.12	6.51
	+35 > Baseline	8.12	22.67
	+60 > Baseline	3.75	21.84
	+60 > +35 minutes	14.37	23.37
Group Difference	**Time Point**	**F**	**p-value**
Lean vs Obese	Baseline	.555	.467
	+35 minutes	3.338	.085

	+ 60 minutes	1.635	.218
	+35 > Baseline	3.52	.078
	+60 > Baseline	1.97	.178
	+60 > +35 minutes	1.844	.192

Asked the question “How hungry do you feel?”, the obese group reported to be less hungry 35 minutes after Exenatide injection versus placebo. The obese group also had a greater decrease in hunger on the drug day (versus placebo day) after eating the meal (versus before the meal). No group differences were observed with the question “how full do you feel?” All scores are drug day minus placebo day.

Table 3. Impact of Exenatide on NTS Functional Connectivity

Contrast:	**Lean**	**Obese**	**Obese> Lean**	**Lean> Obese**	**Lean + Obese**
Region:					
L NTS Functional Connectivity					
L HYP Coordinate		(-6 -4 -12)			
Cluster (Ke)		5			
p-value		.026*			
z-score		3.38			
R HYP Coordinate					
Cluster (Ke)					
p-value					
z-score					
L Thalamus Coordinate		(-20 -36 8)			(-14 -26 16)
Cluster (Ke)		46			13
p-value		.028*			.055
z-score		4.86			3.77
R Thalamus					

Coordinate		(10 -24 12)	(6 -36 4)		
Cluster (Ke)		7	13		
p-value		.054	.108		
z-score		3.79	3.56		
R NTS Functional Connectivity					
L HYP Coordinate					
Cluster (Ke)					
p-value					
z-score					
R HYP Coordinate					
Cluster (Ke)					
p-value					
z-score					
L Thalamus Coordinate	(decreased functional connectivity) (-18 -24 4)	(-10 -16 18)	(-10 -16 16)		
Cluster (Ke)	14	8	51		
p-value	.125	.140	.022*		
z-score	3.51	3.47	4.51		
R Thalamus Coordinate			(8 -8 12)		
Cluster (Ke)			32		
p-value			.022*		
z-score			4.47		

Using the nucleus of the solitary tract (NTS) as a seed, Exenatide administration resulted in greater functional connectivity in the obese group for both right and left seeds in a low frequency band (0.01-0.027 Hz) for hypothalamus and thalamus ROIs. For the left NTS, this change was not seen in the lean group and for the right NTS, this change was greater in the obese than the lean group. Combining groups, greater functional connectivity was observed in the left NTS.

*p-value observed after correction for multiple comparisons. NTS = nucleus of the solitary tract, L = left, R = right.

Table 4. Exenatide Induced NTS Functional Connectivity Correlations with Appetite Measures

A. NTS Functional Connectivity and Calorie Consumption

Group	Region	Coordinate (x,y,z)	Cluster value (K)	p-value (FWE)	Z-score
L NTS Functional Connectivity Correlation with Calorie Consumption					
Obese	L Thalamus	(-20 -36 8)	25	.051	3.74
	R Thalamus	(24 -26 16)	8	.144	3.39
Obese + Lean	L Thalamus	(-18 -36 10)	16	.058	3.69
	R Thalamus	(24 -24 16)	5	.200	3.27
R NTS Functional Connectivity Correlation with Calorie Consumption					
Obese	R Thalamus	(0 -2 4)	20	.126	3.45
Obese > Lean	L Thalamus	(-8 14 16)	16	.078	3.38
	R Thalamus	(6 -6 12)	15	.119	3.47
Obese + Lean	R HYP	(2 -4 8)	11	.008*	3.69
	L Thalamus	(0 -2 2)	9	.030*	3.90
	R Thalamus	(0 -2 2)	32	.064	3.90

All contrasts significant at p.001 uncorrected for Drug-Placebo, RSS3-RSS1 unless otherwise noted

B. NTS Functional Connectivity Correlation with Hunger Ratings

Group	Region	Coordinate (x,y,z)	Cluster value (K)	p-value (FWE)	Z-score
L NTS Functional Connectivity Correlation with Hunger					
Obese	L Thalamus	(-20 -36 8)	69	.024*	4.20
	R Thalamus	(24 -26 16)	17	.054	3.94
Obese + Lean	L Thalamus	(-20 -34 10)	36	.062	3.67
	R Thalamus	(24 -24 16)	9	.107	3.50
R NTS Functional Connectivity Correlation with Hunger					
Lean (decreased correlation)	L Thalamus	(-16 -24 4)	13	.113	3.48
Obese	L Thalamus	(-12 -18 18)	23	.048	3.76
Obese > Lean	L Thalamus	(10 -16 16)	75	.002*	4.76
	R Thalamus	(8 -8 12)	17	.100	3.54
Obese + Lean	R HYP	(4 -2 -6)	4	.022*	3.39
	R Thalamus	(4 -4 -2)	16	.083	3.60

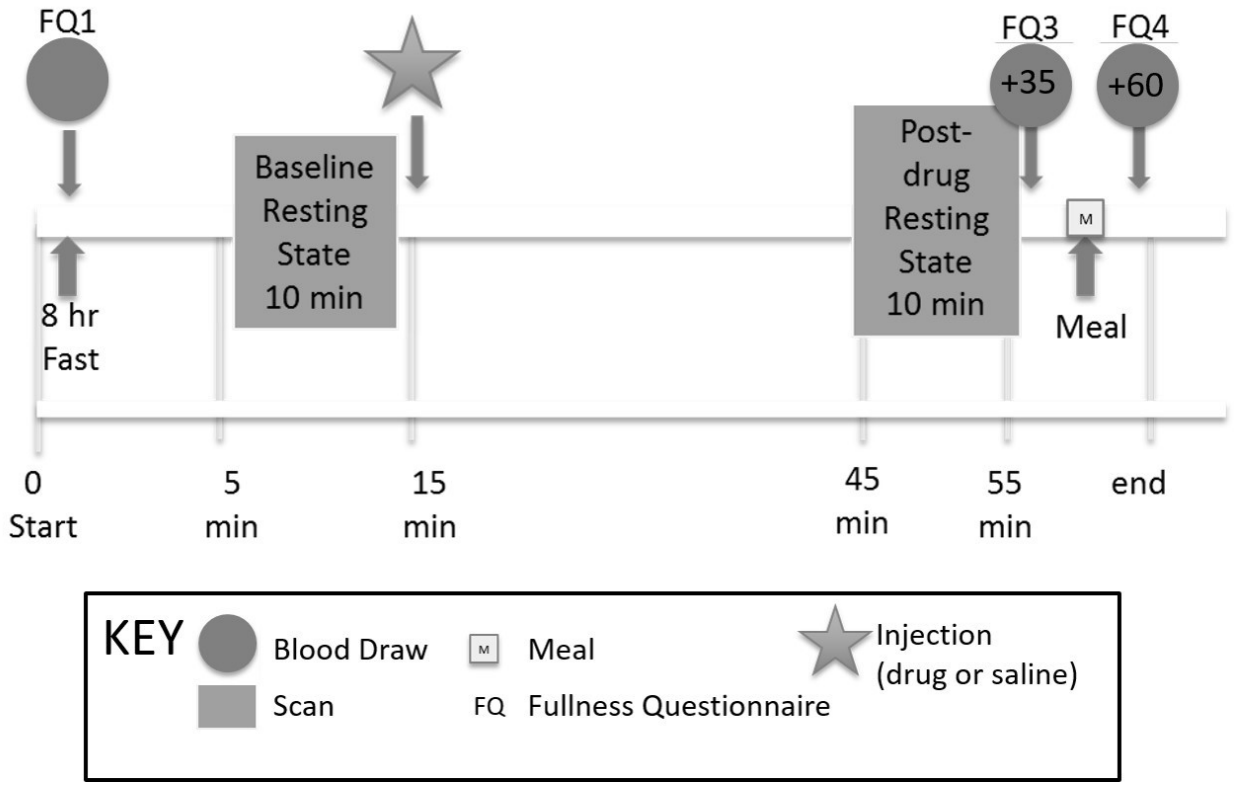
All contrasts significant at p.001 uncorrected for Drug-Placebo, RSS3-RSS1 unless otherwise noted

Functional Connectivity of the nucleus of the solitary tract (NTS) seed with the hypothalamus and thalamus were observed to be correlated with behavioral measures as a result of Exenatide administration. A. Calorie consumption was observed to be correlated with NTS functional connectivity in the obese group only for the left NTS seed and in both groups combined for the right NTS seed. B. Subject hunger ratings were observed to be correlated with NTS functional connectivity in the obese group for the left NTS seed and in the obese group more significantly than in the lean group in the right NTS.

*p-value observed after correction for multiple comparisons. NTS= nucleus of the solitary tract, L=left, R=right

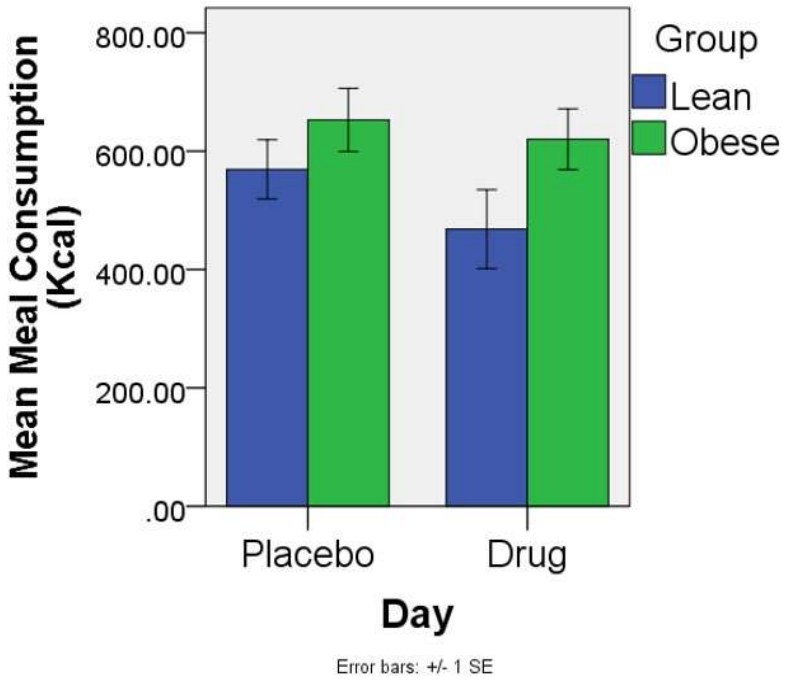
Figures

Figure 1: Basic Scan Day Protocol



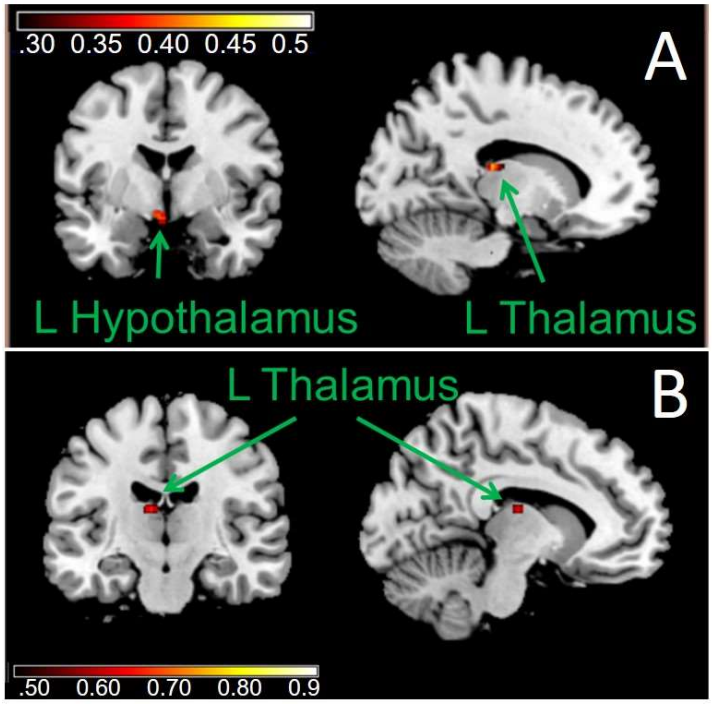
Subjects were brought in after at least 8 hours of fasting. Scans were done both pre and post subcutaneous injection of either drug or saline placebo. After scanning, subjects were given a meal. Blood draws and questionnaires were given before scanning and 35 and 60 minutes after injection.

Figure 2. Calorie Consumption of lean versus obese subjects of a post-scan meal



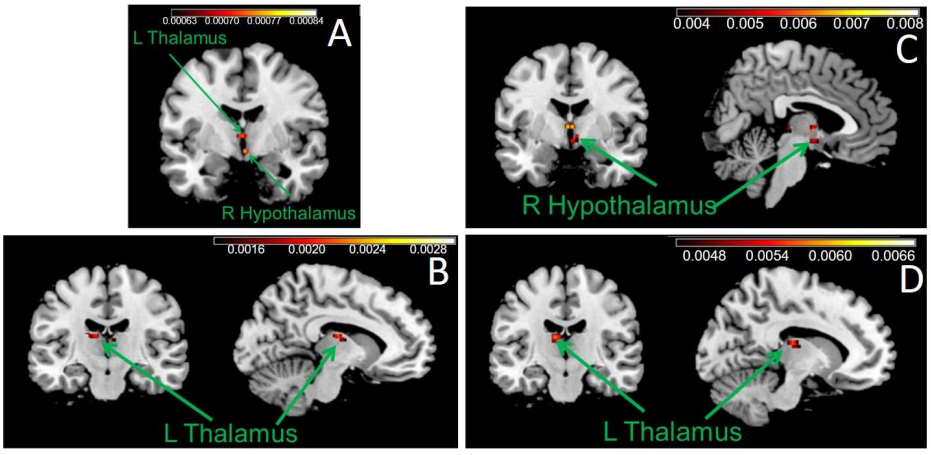
Trends show that both lean and obese groups consumed fewer calories on the day of the Exenatide injection versus the placebo day and that the obese group consumed more calories on both days.

Figure 3A and 3B. Functional Connectivity Analysis of NTS with thalamus and hypothalamus in obese compared to lean



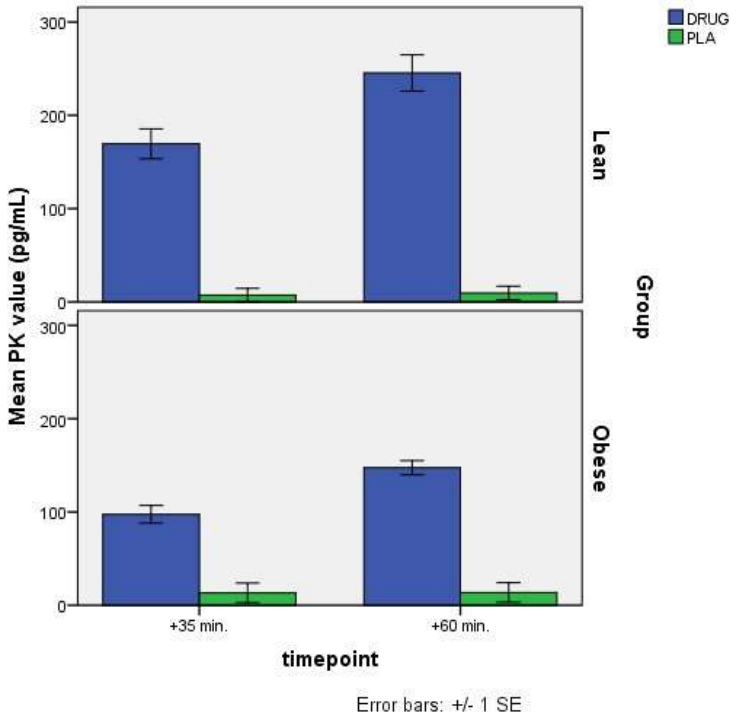
(A)The obese group showed increased left NTS functional connectivity with the left thalamus and the left hypothalamus and (B) compared to the lean group showed increased right NTS functional connectivity with the left thalamus. Color bars reflect t-values.

Figure 4A, 4B, 4C and 4D. NTS Functional Connectivity Correlation Analysis with Calorie Consumption and Hunger



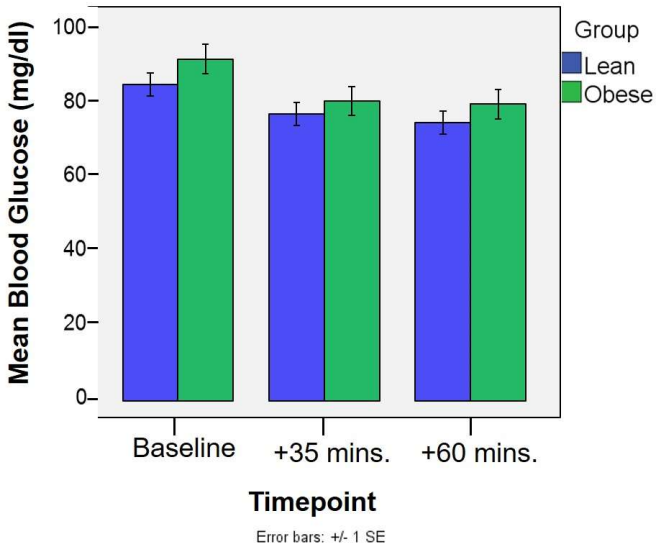
(A) All subjects together showed a positive correlation between calories consumed of a meal and functional connectivity of the right NTS with the right hypothalamus and left thalamus. (B) At baseline before scanning, the lean group showed a greater correlation between hunger and right NTS functional connectivity with the left thalamus. After Exenatide injection (versus placebo), (C) all subjects combined had a correlation between hunger and right NTS functional connectivity with the right hypothalamus and (D) the obese group had a greater correlation between hunger and right NTS functional connectivity with the left thalamus. Color bars reflect t-values.

Supplemental Figure 1: Post-Injection PK Values



Both lean and obese groups had significantly greater levels of Exenatide in the blood on the day of Exenatide injection compared to placebo injection. They also both had greater levels of Exenatide 60 minutes post injection versus 35 minutes post injection. Comparing groups, the lean group had greater levels of Exenatide in their bloodstream both at 35 (p.=.004) and 60 (p=.004) minutes after injection. *p>.05 **p>.001.

Supplemental Figure 2 : Mean Glucose Blood Levels



Both lean and obese groups had significantly reduced levels of mean blood glucose as a result of Exenatide both 35 (lean p=0.026, obese p=0.007) and 60 (lean p=0.030, obese p=0.004) minutes post-injection compared to a pre-injection baseline. There were no significant differences between the lean and obese groups at any timepoint.

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CHAPTER SEVEN

General Conclusions, Major Limitations and Future Perspectives

General Conclusions

Dysregulation of Homeostatic and Hedonic Networks in Obesity

It has been theorized that in obesity, there is an imbalance between brain networks involved in homeostatic and reward related signaling. While not the first studies to shows this possibility, the series of studies in this dissertation provides further evidence that a brain dysregulation associated with obesity exists, and that obese individuals respond differently to a drug which stimulates the actions of a known satiety hormone.

This dysregulation was observed firstly with the viewing of food pictures after consuming a calorie beverage. Although the obese group reported reduced behavioral response, they demonstrated a greater brain response compared to the lean subjects. In particular, there seemed to be a disconnect between appetite-related questionnaire response and brain response when it came to hedonic activity, with the obese group reporting less satisfied by a caloric drink, yet indicating greater reward or craving in the brain with the viewing of food images. Beyond a reward network, this disparity could reflect abnormalities in brain networks involved in translating a reward response to a change in eating behavior. This disconnect with behavior was also observed in the drug study where obese women showed a trend to eat more despite reporting to be less hungry after the drug injection.

A difference in the interpretation of reward response was also observed in obese subjects at rest (without the food images prompt). Both analyses examining grey matter volume of the nucleus accumbens as well as frequency fluctuations and functional connectivity in a resting brain, demonstrated differences between groups of healthy obese and lean women, particularly in a nucleus accumbens-based hedonic network. The increase in frequency fluctuations and in functional connectivity observed in the obese group compared to the lean suggest a clear dysfunction in hedonic circuitry. These patterns in brain activation are similar to another

disorder known for having an imbalance or over-activation of hedonic drives – addiction, to be discussed in another section.

Although our studies cannot draw firm conclusions on the physiological significance of a larger nucleus accumbens volume in obese individuals, brain volume changes have been linked to changes in neuronal activity as well as changes in an inflammatory response (1, 2). Both of these options reflect potential mechanisms by which brain abnormalities occur in the obese. Certain regions of the brain have also been shown to fluctuate in size in response to changes in hormonal regulation, for example, as seen throughout a woman's menstrual cycle (3). Whether the changes are a result of degree in food intake or adipocyte signaling, it is not known how easily these changes may occur or how often they might fluctuate.

While examining a baseline resting state suggests a difference in a nucleus-accumbens based hedonic network, our study examining the impact of Exenatide, points to obesity-related abnormalities in an NTS-based homeostatic network. The drug, known to have an impact on satiety, altered the functional connectivity of regions associated with a homeostatic network with an increased functional connectivity observed in the obese group. This finding could suggest simply that Exenatide modulates homeostatic regulation of food intake differently in obese women. It could also suggest that the drug might be working to increase the engagement of a homeostatic network in a generally less sensitive system. This possibility supports the concept that obese subjects are less responsive to homeostatic signals about energy needs or that obese subjects have more resistance to satiety signals. Exenatide activates GLP-1 receptors so the increased of engagement of this NTS-based network as a result of the drug injection could reflect a baseline decrease in GLP-1 or GLP-1 receptor activation in our obese subjects. A more accurate assessment of GLP-1 receptor activity and any potential changes due to an exogenous GLP-1 receptor agonist could be done looking at receptor mRNA expression and positron emission tomography (PET) scans.

The drug-induced increase in functional connectivity was also associated with appetite-related behavioral measures including calorie consumption and hunger, suggesting a link between the brain changes and eating behaviors. All subjects showed these correlations but the obese had an even greater correlation between reported hunger and the NTS functional connectivity with the thalamus suggesting that perhaps this strengthening of a depressed engagement of a homeostatic network impacts the feelings of hunger in obese individuals.

Taken together, our studies' results suggest that perhaps the obese women's brain have an imbalance between hedonic and homeostatic networks. More specifically, we observed an increased response of a hedonic network reflective of altered interpretation of reward and our drug study suggested that our obese subjects had a reduced homeostatic response reflective of perhaps a less sensitive homeostatic network. It is hard to speculate whether this imbalance is a cause of, or result from having an increased BMI, but it is clear that obesity comes with abnormal brain networks. Future studies might tease this apart by delving into more longitudinal questions, examining the brain throughout development or before and after a successful weight loss program or bariatric surgery to see if and how these brain networks might change, or not, along with BMI.

Parallels Between Obesity and Addiction

Addiction, particularly drug addiction, has been well studied, and is known to impact hedonic networks in the brain related to reward and reward-processing. While food addiction is an acknowledged clinical condition, only a small percentage of people who are obese test to have a food addiction via the Yale Food Addiction Scale (4). The work in this dissertation suggests that at least on the level of brain organization, parallels between obesity and drug addiction might extend beyond those who have a verified addiction to food.

For our studies, when looking at images of palatable food, obese women had a greater response in the brain of a network which included regions associated with reward than a lean group. This network was even more responsive after the consumption of a high calorie sucrose drink (versus a non-nutrient lower calorie drink). Animals studies have suggested that mice can respond to sugar in a similar way as they respond to other drugs of addiction (5). Increased sugar consumption can be associated with obesity as well as functional connectivity changes in regions of reward in the brain (6), and could provide a valuable link to a potential cause of obesity. Studies in animals could help to explore some of the physiological mechanisms involved in obesity that might be similar to addiction as well as explain some of the observations made in our study looking at the impact of a sweet drink on obese women's brains.

Our resting state studies provide further similarities to studies of addiction. The increased frequency fluctuations in the resting brain of our obese group could potentially suggest a decrease in neuronal activity in these reward-related regions. This could reflect the same impairment in the dopamine system thought to underlie the pathophysiology of addiction. In drug addiction, the increase in intake of the drug results in a loss of dopamine 2 receptors in the nucleus accumbens which can disrupt the hedonic response. This reduced number of dopamine 2 receptors has also been shown in obesity (7), suggesting an increase of food intake could be interpreted in the brain in a similar manner to a drug addiction.

Our studies also observed an increase in functional connectivity between the nucleus accumbens and cortical areas of the brain for the obese subjects. This increase has also been observed in studies on drug and nicotine addicts and has been suggested to relate to issues with control and compulsivity (8, 9, 10). These resting stage differences in the obese women's brain compared to lean can provide several different insights but none as exciting as the potential link to types of addiction that are more well studied.

Brain imaging studies can provide much information but in order to examine more mechanistic or physiological underpinnings of a disorder, different approaches of study need to be included. Drug addiction studies have provided scientists with a wealth of information on all levels of study. If parallels can be made between obesity and drug addiction at the level of the brain, it might reflect a deeper connection between what is going on down to the level of the receptor or over to the level of behavior and behavior modification. Drug addiction treatment is also quite well studied and might provide some valuable insights when it comes to looking at potential treatments for obesity.

Using the Brain in Clinical Assessment and Treatment of Obesity

Obesity can be defined in many different ways including Body Mass Index (BMI), hip-waist ratio, and percent body fat composition. Obesity can be viewed as an eating disorder, an addiction, or simply as something that is co-morbid with other disorders such as diabetes or cardiovascular disease. Especially considering the variety of causes and presentations of obesity, the clinical assessment of what obesity is and how it should be viewed in health is not as clear cut as it has been described in the past.

The brain, particularly the resting brain, could be a valuable resource as a potential neurobiological biomarker for obesity as it allows for easy clinical assessment without any visual or task component. Also, the types of analyses we explored in the studies discussed in this dissertation could play a role in determining any variants of obesity and what it might mean for an individual's health. These analyses could easily be expanded to look at other factors that might influence the brain in obesity, including genetics, personality factors, or other seemingly unrelated individual diagnoses. Examining the resting brain and any intervention-induced

alterations might also be critical in assessing the effectiveness of various potential treatments of obesity.

Exenatide itself has been explored in use for weight-loss treatment (11, 12). Although, due to side effects, the drug itself will probably not come on the market for that purpose, it still might play a role in future treatment options either as a component of a multivariate treatment option or as a model for other drugs that would not have the same side effects. Knowing how Exenatide impacts the brain could provide valuable insights not just in unveiling how the drug works to impact the brain in obesity but also as a potential brain-reflective benchmark for other potential treatments.

Study Limitations

Limited sample size, measurements not taken or observed, and restrictions of analysis type are limitations for all of the studies mentioned within this dissertation. The study of homeostatic and hedonic networks upon viewing of food images after consumption of non-nutrient or sucrose beverages provided many insights, but was limited by a lack of a control group who didn't consume a beverage, and a failure to take into account body size when determining the quantity of beverage given. The study would have also benefited from additional measurements such as blood glucose levels and an increase in sample size, as several analyses did not have enough power to push certain results to a level of significance.

Examining baseline resting scans revealed obesity-related differences, but this study was limited by a lack of standardized measurements including autonomic measures known to have an impact on resting state, and behavioral questionnaires that could have provided further insight to how the brain differences may be correlated with other factors such as behavior

related to food intake. There is also limited information on the interpretation of frequency band oscillations and their physiological significance.

The Exenatide study is severely limited by the number of subjects examined. An increased sample size would have allowed for testing of any potential individual variants in the causes of obesity which would provide greater information about populations within an obese subject group that may be impacted differently by the drug. This study would have also benefited from a better way to test obesity based on fat composition and by taking into account the amount of subcutaneous fat to determine perhaps a more appropriate dosage of the drug. Finally, for any similar resting state study, further studies or analysis types might also look at the interaction between hedonic and homeostatic networks in their relation to obesity and food intake.

Future Perspectives

Obesity Study Expansions

The results presented within this dissertation could be seen as a great jumping off point for several different areas of study. A larger sample of healthy obese women would provide the opportunity for similar analyses to provide even more information including the exploration of different subtypes within an obese population, and how brain alterations might change based on factors such as genetics, personality type, food preferences, environment, or a number of other potentially differentiating factors. It would also be informative to gather a sample of obese individuals that is more reflective of the general population, even including men in order to investigate sex differences when it comes to obesity and the brain.

Our conclusions bring up the question of what is addiction in obesity. This is a topic that needs much further exploration to determine if we can lump obesity into a greater addiction category,

or if it perhaps needs a different definition to describe a similar pattern of altered reward networks in the brain. What exactly are the parallels and can we parse out some differences between a food-related addiction and drug addiction?

Finally, a greater sample of subjects given the Exenatide drug could give our analyses more power to explore more whole brain results, to look further at the hedonic network's response to the drug and to investigate any cross talk (or lack thereof) between homeostatic and hedonic brain regions.

Looking at Resting State Changes in Bariatric Surgery

One of the most successful and long lasting treatments of obesity is bariatric surgery. The shortening of the intestinal tract or reduction of stomach size through banding or stapling have all proven to have an impact on BMI reduction. Although the most proven treatment, bariatric surgery is not effective for everyone, especially in the long term. Brain imaging, and in particularly resting state analyses, could prove a useful tool in defining the impact of these types of surgeries as well as determining any qualities that may suggest in advance of the surgery if the person is a good candidate for successful and long-lasting weight reduction.

The next step in our ongoing studies of obesity and the brain is to use some of the brain imaging techniques we have been utilizing to undergo a long term study to examine the impact of bariatric surgery on the obese women's brain. The study will look at subjects before, as well as 3, 6, and 12 months after bariatric surgery. At each visit subjects will undergo a fMRI picture viewing paradigm, DTI, and resting scans, as well as answer questions about their feelings of hunger and satiety. The aims of this study are to characterize brain mechanisms underlying the effectiveness of bariatric surgery and to answer the question whether brain activity patterns can be used as predictors of adequate weight loss following the surgery.

The more general hypotheses of this study are: 1) Brain activity, connectivity patterns and white matter connections associated with subjective sensations of hunger and satiety will change after bariatric surgery and these changes will be associated with weight loss, 2) Subjective feelings of hunger and satiety and the brain representations of these sensations differ between patients who achieve adequate weight loss after bariatric surgery vs patients who do not, and 3) Resting state brain activity and evoked brain response to food and food cues can be used as moderators of treatment outcome (ie may predict success after bariatric surgery). For example, perhaps patients who show an abnormal hedonic response, represented by increased activity in hedonic regions such as the nucleus accumbens, in response to particular food images, or a subgroup who demonstrate a lack of functional connectivity between homeostatic and hedonic brain regions in their resting state scans will be identified pre-surgery to have less of a successful long-term response post-surgery.

The hope with this study and potential future studies is to continue to use these brain imaging techniques to explore the differences in both homeostatic and hedonic brain networks in the obese female brain with the goal of further defining differences, individual variations based on a variety of clinical assessments, and the effectiveness of potential treatment options.

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