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Obesity is associated with altered mid-insula functional connectivity to limbic regions underlying appetitive responses to foods.

Jason A. Avery¹, Joshua N. Powell^{1,2,3}, Florence J. Breslin^{1,2}, Rebecca J. Lepping⁴, Laura E. Martin⁴, Trisha M. Patrician⁵, Joseph E. Donnelly⁶, Cary R. Savage^{2,6}, and W. Kyle Simmons^{1,7}

¹Laureate Institute for Brain Research, Tulsa, OK

²Center for Health Behavior Neuroscience, University of Kansas Medical Center, Kansas City, KS

³Graduate School of Social Work, University of Denver, Denver, CO

⁴Hoglund Brain Imaging Center, University of Kansas Medical Center, Kansas City, KS

⁵Hennepin County Medical Center, Minneapolis, MN

⁶Center for Physical Activity and Weight Management, University of Kansas Medical Center, Kansas City, KS

⁷Banner Alzheimer's Institute, Phoenix, AZ

Abstract

Obesity is fundamentally a disorder of energy balance. In obese individuals, more energy is consumed than is expended, leading to excessive weight gain through the accumulation of adipose tissue. Complications arising from obesity, including cardiovascular disease, elevated peripheral inflammation, and the development of Type II diabetes, make obesity one of the leading preventable causes of morbidity and mortality (Johnson et al., 2014). Thus it is of paramount importance to both individual and public health that we understand the neural circuitry underlying the behavioral regulation of energy balance. To this end, we sought to examine obesity-related differences in the resting state functional connectivity of the dorsal mid-insula, a region of gustatory and interoceptive cortex associated with homeostatically-sensitive responses to food stimuli (Avery et al., 2017; Avery et al., 2015; Simmons et al., 2013b).

Within the present study, obese and healthy weight individuals completed resting fMRI scans during varying interoceptive states, both while fasting and after a standardized meal. We examined group differences in the pre vs. post-meal functional connectivity of the mid-insula, and how those differences were related to differences in self-reported hunger ratings and ratings of meal pleasantness.

Obese and healthy weight individuals exhibited opposing patterns of eating-related functional connectivity between the dorsal mid-insula and multiple brain regions involved in reward,

valuation, and satiety, including the medial orbitofrontal cortex, the dorsal striatum, and the ventral striatum. In particular, healthy weight participants exhibited a significant positive relationship between changes in hunger and changes in medial orbitofrontal functional connectivity, while obese participants exhibited a complementary negative relationship between hunger and ventral striatum connectivity to the mid-insula. These obesity-related alterations in dorsal mid-insula functional connectivity patterns may signify a fundamental difference in the experience of food motivation in obese individuals, wherein approach behavior toward food is guided more by reward-seeking than by homeostatically-relevant interoceptive information from the body.

Keywords

Insular cortex; striatum; obesity; functional connectivity; reward; homeostasis

INTRODUCTION

Adaptive regulation of food intake requires that peripheral signals related to hunger and satiety be used to guide appetitive responses to food. Heretofore, neuroscientific studies of appetite have mainly focused on the role of circulating energy-signaling molecules that cross the blood brain barrier and bind to receptors on hypothalamic neurons, which in turn project to limbic and reward regions, thereby either enhancing or inhibiting appetite (Klok et al., 2007; Coll et al., 2007). Although these hypothalamic signaling pathways are certainly important to the regulation of food intake, comparatively little attention has been paid to an alternate pathway by which the peripheral body signals the brain: the vagal afferent interoceptive system, and its integration with reward neurocircuitry. This lack of attention is somewhat surprising given involvement of the insula, the cortical target of the vagal afferent pathway, in both gustatory and interoceptive processing (Craig, 2002; Small, 2010), as well as evidence (described below) that the insula's response to food cues is modulated by peripheral markers of energy signaling.

Peripheral signals of energy availability and interoceptive signals about gastric volume first reach the cortex via the vagal afferent pathway, which relays signals through the brainstem, thalamus, and on to the insular cortex (Craig, 2002; Pritchard et al., 1986; Beckstead et al., 1980). Human neuroimaging research has demonstrated activation of the insula associated with gastric distension as well as interoceptive attention to stomach sensations (Simmons et al., 2013a; Wang et al., 2008). Gustatory signals follow along a similar pathway from the periphery as visceral signals, traveling from the brainstem to then gustatory thalamic nuclei, and on to the insula/frontal operculum (Beckstead et al., 1980; Pritchard et al., 1986). Neuroimaging studies in humans have likewise demonstrated insula activation to both the sight and taste of food (Simmons et al., 2005; van der Laan et al., 2011; Veldhuizen et al., 2011; Malik et al., 2008; Uher et al., 2006), with some studies reporting overlapping activation for taste responses and visual food cues within the same insula regions (Simmons et al., 2013b). This same study identified a region of the human gustatory cortex in the dorsal mid-insula that exhibited category specific activation to food vs. object pictures, exhibiting a response to food pictures that is negatively modulated by levels of circulating glucose, suggesting the involvement of this region in the homeostatic response to food

stimuli (Simmons et al., 2013b). This finding is consistent with other studies demonstrating that the activation of this region is modulated by circulating glucose and insulin levels (Kroemer et al., 2013; Page et al., 2011; Wright et al., 2016) and in response to ingestion of various macronutrients (Li et al., 2012). Related research in rodents and humans has indicated that the mid-insula also exhibits overlapping responsiveness for interoceptive and gustatory stimuli (Avery et al., 2015; Simmons et al., 2013a; Simmons et al., 2013b; Avery et al., 2017; Ogawa and Wang, 2002; Hanamori et al., 1998), and therefore may serve to integrate taste-related information with interoceptive information of the body's homeostatic needs. Indeed, some theories of the insular gustatory cortex posit that it functions as a nutrient-responsive integrated circuit, regulating the behavioral response to nutritive stimuli, according to the body's homeostatic state (de Araujo et al., 2012). Given its connectivity to cortical and subcortical limbic structures involved in reward and appetitive behaviors such as the amygdala, the striatum, and the orbitofrontal cortex (Mesulam and Mufson, 1982; Scott and Plata-Salaman, 1999; Rolls, 2015), the mid-insula would seem to be well-positioned within the brain to serve as a conduit for passing along interoceptive information about the state of the body to those regions, thereby supporting adaptive (allostatic) behaviors that allow humans to meet their energy needs and thereby maintain homeostasis (Barrett and Simmons, 2015; Kleckner et al., 2017).

If indeed the dorsal mid-insula plays an important role in integrating food-related interoceptive signals and delivering that information to the appetitive and reward-related regions to help regulate food intake, then we would predict that obesity is associated with abnormal function of the dorsal mid-insula under varying interoceptive states. To test this possibility, we examined the functional connectivity of the dorsal mid-insula during different interoceptive states (before and after eating a meal) using resting-state fMRI scans of obese and healthy weight adults. In particular, we first sought to determine whether obese and healthy weight participants exhibit different dorsal mid-insula functional connectivity profiles before and after eating a meal, and whether these differences relate to how appetizing the subjects experienced the meal to be. Second, we sought to determine whether obese and healthy weight participants exhibited differences in the relationship between dorsal mid-insula functional connectivity and the change in their interoceptive self-reports of hunger experienced before versus after eating the meal.

Methods

Subjects—(52) obese participants (33 female) and (18) healthy weight participants (14 female) that took part in a behavioral weight loss clinical trial [NIH DK080090; NCT02031848] (Szabo-Reed et al., 2015) and agreed to undergo fMRI scanning. All fMRI scanning sessions within the present study occurred during the baseline phase of each subjects' participation within the weight loss trial. Participants were recruited via advertisements, and a university-based weight management center. Inclusion criteria for obese participants included a BMI between 30–45 kg/m², and a BMI between 18–25 kg/m² for healthy weight participants. Special diets (e.g., vegetarian and Atkins), appetite or metabolic medications (e.g., thyroid, beta blockers, and Meridia), smoking, and diabetes were exclusions for the obese participants. Additional exclusion criteria for both groups

included current eating disorder, current major depression, history of neurological disease, pregnancy within the past 6 months, cancer, heart disease, and contraindications for MRI (e.g., metal implants). All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Human Subjects Committee of the University of Kansas Medical Center. Informed consent was obtained from all individual participants included in the study.

Experimental Design

Participants' baseline fMRI appointments lasted approximately 4 to 5 hours, between the hours of 10am and 3pm. During those appointments, participants completed behavioral assessments, safety screenings, a physical examination, a short meal, and two 45-minute fMRI scans (Szabo-Reed et al., 2015). To investigate changes that accompany typical mealtime eating behavior, participants were scanned while hungry (Pre-meal; following at least a 4-hour fast) and after eating a small lunch that was standardized for energy [Kcal = 500] and macronutrient content. Participants that arrived fasted would complete the first (pre-meal) fMRI scan, eat the standardized meal, complete the second (post-meal) fMRI scan, and then complete all remaining testing. Other participants would eat the standardized meal upon arrival, complete the post-meal fMRI, and then wait 4-hours before completing the fasted-fMRI (pre-meal); during which time, all other evaluations were completed. Order of pre-meal and post-meal scans was counterbalanced across participants, to account for potential order effects. Prior to eating, participants were weighed and their height was recorded. Immediately prior to each of the scans (pre-meal and post-meal), participants were asked to rate their levels of hunger, thirst, fullness, and discomfort on a visual analog scale (VAS) ranging from 1 to 100. After receiving the meal, participants also rated on a VAS (1 – 100 scale) how appetizing they found the meal. The resting-state scan (6 min 36 s) followed a structural scan and two functional scans while passively viewing food and animal pictures (Bruce et al., 2014; Bruce et al., 2012; Holsen et al., 2005). Participants were instructed to close their eyes during the resting-state scan. The entire scanning session lasted 45 min.

Imaging Parameters

Data were acquired with a 3-Tesla Siemens Allegra, head-only MRI scanner. To minimize susceptibility artifact in ventromedial prefrontal regions, participants were positioned so that the angle of the AC–PC plane was between 17 and 22 degrees in scanner coordinate space. T1-weighted anatomic images were acquired with a 3D MPRAGE sequence (repetition time (TR)/echo time (TE) = 23/4 ms, flip angle = 8 degrees, field of view = 192 mm, matrix = 192×192 mm², 208 slices, slice thickness = 1 mm). Resting-state gradient echo scans were acquired in 43 contiguous axial slices at an angle of 40 degrees to the AC–PC line (TR/TE 3,000/30 ms, slice thickness = 3 mm [0.5 mm skip], in-plane resolution = 3mm × 3mm, 130 volumes).

fMRI-Image Processing

Preprocessing of the resting-state scans was performed using a modified version of the ANATICOR method (Jo et al., 2010), implemented through the AFNI program `afni_restproc.py`. The first 4 volumes of the resting state-scan were excluded to allow the

fMRI signal to reach steady state. Following this, a de-spiking interpolation algorithm (AFNI's 3dDespike) was used to remove transient signal spikes from the EPI data. The volumes were slice time corrected and co-registered to the first volume (which was registered to the anatomical scan). Masks of the subject's ventricles and white matter were constructed using the subject's anatomical scan and the AFNI program 3dSeg. The average time course during the resting-state scan was extracted from the ventricle mask and subsequently used to account for any components of the MR signal due to cerebrospinal fluid in the ventricles. Next, local physiological noise present in white matter was estimated using the AFNI program 3dLocalstat, which calculated the average signal time-course for all white matter voxels within a 1.5 cm radius of each gray matter voxel. Additional regressors of non-interest were constructed from the mean, linear, quadratic, and cubic signal trends, as well as the 6 normalized motion parameters (3 translations, 3 rotations) computed during the image registration preprocessing. The predicted time-course for these nuisance variables was constructed using AFNI's 3dTfitter program and then subtracted from each resting-state voxel time-course, yielding a residual time-course for each voxel. The residual images were smoothed with a 6mm FWHM Gaussian kernel, resampled to a $2 \times 2 \times 2$ mm³ grid, and spatially transformed to stereotaxic space conforming to the Talairach and Tournoux Atlas.

Further motion correction procedures (i.e., scrubbing) were utilized to reduce false group differences due to uncontrolled subject motion (Power et al., 2012). The six motion parameters from the image registration process were used to construct a time series reflecting the Euclidean normalized derivative of the motion. This time series was thresholded so that any time point where the derivative was greater than 0.3 (roughly 0.3 mm motion) was censored. We also censored any time point where more than 5% of brain voxels were considered outliers (3dToutcount). Time points censored by the union of both methods were removed in the subsequent regression analysis.

Analyses

We functionally defined the dorsal mid-insula seed region using data from prior published studies with a different cohort of subjects. The seed region was a mask composed of the intersection of dorsal mid-insula voxels shown to be selective both for interoceptive attention to the viscera (Simmons et al., 2013a), as well as responsive to gustatory stimulation and food pictures (Simmons et al., 2013b)(Figure 1). At the subject level, the dorsal mid-insula cortex (dmIC) time series was constructed for both pre and post meal scans separately by calculating the average resting-state time series over the voxels within the dorsal mid-insula intersection mask. Using a multiple regression approach, we produced a map of the correlations (r-values) between the dmIC time-series and the rest of the brain. These r-value maps were then fisher-transformed into z-score maps.

At the group level, the z-score maps were used to examine group differences in dmIC functional connectivity pre-meal vs. post-meal. We used the AFNI program 3dttest++ to implement a 2×2 [Group (Obese - Healthy Weight) $\times$ State (pre meal – post meal)] analysis comparing the change in dmIC functional connectivity between Obese and Healthy Weight participants. Within ROIs identified in this analysis, we extracted z-score values from

individual subject pre and post meal z-score maps, to identify specific effects underlying observed interaction effects.

The AFNI program 3dttest++ was also used to examine the relationship between changing dmIC functional connectivity and change in participants' subjective ratings of hunger. Separate one-sample paired t-tests of dmIC z-score maps were conducted for both groups, using the difference in participants pre-scan hunger ratings (pre-meal – post-meal) as a covariate. Follow-up ROI analyses were performed by extraction of z-score parameters using AFNI's 3dMaskAve program. Resulting statistical analyses were performed in the R software package.

Multiple Comparisons Correction—The T-statistic maps derived from the analyses of resting-state data in the present study were separately corrected for multiple comparisons using cluster-size FWE correction within *a-priori*-defined anatomical regions. An initial voxel-wise *p*-value of 0.005 was applied to the statistical maps. The group-level statistical maps were then small-volume corrected (SVC) for multiple comparisons within those *a-priori*-defined regions at $p < 0.05$ using non-parametric permutation tests, as follows:

In order to limit the number of search volumes used within the SVC approach, we constructed a single spatially contiguous mask from the union of all of the anatomical regions of interest. As we were primarily interested in connectivity between the insular cortex and other brain regions involved in homeostatic and reward processing, the mask itself consisted of both cortical regions, (orbitofrontal and insular cortices), as well as sub-cortical regions, (corpus striatum, amygdala, thalamus, and hypothalamus), involved in visceral sensory processing, reward, and appetitive responses to food. These regions were chosen based on their well-established roles in the neurocircuitry involved in interoceptive awareness of the body (Craig, 2002), reward processing (Berridge and Kringelbach, 2015), the generation of pleasantness inferences (Simmons et al., 2014), and addiction (Koob and Volkow, 2010). Anatomical masks of these regions were selected from a pre-rendered stereotaxic brain atlas available within AFNI (DD Desai Maximum Probability Map Atlas), based on probability maps generated for 35 cortical areas (Desikan et al., 2006) and the parcellation of cortical and subcortical structures (of the AFNI Talairach N27 atlas brain) generated by the FreeSurfer program. This atlas is freely available for reference in the AFNI software distribution (<http://afni.nimh.nih.gov>). The 'limbic/appetitive' mask that resulted from the union of these anatomical masks, is a spatially contiguous volume of approximately 136,000 mm³ in size.

Evidence from recent analyses of cluster thresholding methods used in fMRI studies (Eklund et al., 2016) indicates that many family-wise error correction methods using parametric cluster-size simulation techniques generate an exaggerated number of false positive results. As a result, cluster-size correction for multiple comparison was performed using non-parametric permutation tests, which incorporates an empirical (rather than a theoretical) distribution of cluster sizes generated by random permutations of the input data. Within the limbic mask, these corrections were implemented in the FSL program 'randomise' (Winkler et al., 2014). Surviving clusters were corrected at a cluster-size $p < 0.05$, using an initial cluster-defining threshold (CDT) of $p < 0.005$. The *p*-value assigned

to these clusters represents the probability that a cluster of that size or larger would appear by random, out of a cluster-size distribution generated from 1000 random permutations of the input datasets.

Results

Behavioral Results

No significant differences between groups were observed for age [$p = 0.37$], education [$X^2(4) = 4.8$; $p = 0.31$], sex [$X^2(1) = 1.17$; $p = 0.28$], or depressive symptoms as measured by the Center for Epidemiologic Studies Depression (CESD) scale [$p = 0.18$]. Additional demographic and anthropometric data are included in Table I. Obese and healthy participants did not differ in how appetizing they rated the meal received prior to their post-meal scan [$p = 0.61$]. Participants' ratings of hunger, thirst, and fullness differed significantly pre to post-meal ($p < 0.001$; Table II). Obese and healthy weight participants did not differ in their behavioral ratings overall, nor did they differ in the pre vs. post-meal difference of those ratings (Table II). However, the group by state effect on fullness ratings did trend toward significance ($p < 0.06$), as the post-meal increase in fullness ratings was numerically larger for healthy weight than obese participants (47.7 vs. 32.2), indicating that the standardized meal was not quite as filling for obese participants as it was for healthy weight participants.

Resting-State Functional Connectivity Results

Dorsal Mid-insula functional connectivity between groups and meal

appetitiveness—A group (OB vs HW) by state (pre vs post meal) interaction effect was observed for dorsal mid-insula functional connectivity to the right dorsal striatum, in a cluster which included the dorsolateral caudate and putamen (Figure 2, Table III). A planned follow-up ROI analysis within this region to examine the specific effects underlying the interaction identified that A) obese participants exhibited a significant increase in functional connectivity between scans, whereas B) healthy weight participants exhibited a significant decrease in functional connectivity between scans, and C) during both pre and post-meal scans, obese and healthy weight participants exhibited significant differences in resting-state functional connectivity between the dorsal mid-insula and dorsal striatum (Figure 2). An additional analysis of co-variance (group $\times$ state $\times$ pleasantness) identified that obese and healthy participants also differed significantly in the relationship between functional connectivity change (RSFC) and meal pleasantness ratings. Among obese participants, those who rated their meals as more appetizing exhibited significantly increased connectivity between the mid-insula and the dorsal striatum ($r(50) = 0.43$, $p = 0.001$; Figure 2). Conversely, healthy weight participants who rated meals as more appetizing exhibited decreased functional connectivity between these regions ($r(16) = 0.56$, $p = 0.01$). We subsequently performed a linear regression analysis to identify if the relationship between appetitiveness ratings and insulo-striatal connectivity differed between groups. This analysis confirmed that the slopes of these relationships differed significantly between groups ($t(66) = 4.36$, $p < 0.001$; Figure 2).

Dorsal Mid-insula connectivity and hunger—Obese and healthy weight participants also exhibited divergent relationships between differences in mid-insula functional connectivity and differences in hunger, pre vs. post-meal. Healthy weight participants exhibited a significant relationship between change in hunger and change in functional connectivity to a region of the left orbitofrontal cortex (OFC), approximately located on the medial orbital sulcus, on the border between Brodmann Areas 11m and 11l, anterior to the genu of the corpus callosum (Figure 3, Table IV). In these participants, hunger and insula-OFC functional connectivity were positively related. As hunger ratings decreased post-meal, so too did connectivity between the OFC and the dorsal mid-insula. This relationship was not observed in obese subjects at the voxel-wise level, nor at the ROI level ($r(50) = 0.00$, $p = 0.99$; Figure 3). In contrast, obese participants exhibited a significant relationship between change in hunger and change in functional connectivity to the left ventral striatum, in a region stretching from the head of the caudate nucleus into the accumbens area. In these participants, hunger and ventral striatal functional connectivity were negatively related. Specifically, the greater the decrease in hunger post-meal, the greater the increase in connectivity between mid-insula and the ventral striatum (Figure 3, Table IV). This relationship was not observed in healthy weight subjects at the voxel-wise level, nor at the ROI level ($r(16) = 0.01$, $p = 0.97$; Figure 3).

DISCUSSION

Prior studies have shown that the insula is engaged in both food-related processing and awareness of homeostatic interoceptive signals of hunger and satiety, including responses to food stimuli that are related to circulating markers of energy availability (Kroemer et al., 2013; Page et al., 2011; Simmons et al., 2013b; Wright et al., 2016). Additionally, various theories about the insula's role in cognition and behavior assert that it distributes information about the interoceptive state of the body to the wider brain to guide behaviors that support energy homeostasis (Barrett and Simmons, 2015; Craig, 2009; Seth and Friston, 2016). Given that obesity most often arises from chronic excess energy intake relative to an individual's homeostatic needs, we reasoned that under varying interoceptive states (e.g., pre- vs. post-meal) obese adults might exhibit abnormal coupling between the insula and other brain regions involved in appetite and eating behavior.

Within the present study, obese and healthy weight individuals completed resting fMRI scans, both while fasting and after a standardized lunch. The analyses within this study were performed to examine whether obese adults under states of hunger and satiety exhibit altered functional connectivity of the dorsal mid-insula, the region within the insula that has been associated with the convergent processing of both gustatory and visceral interoceptive information (Avery et al., 2017; Avery et al., 2015), as well as metabolic modulation of responses to food pictures (Kroemer et al., 2013; Page et al., 2011; Simmons et al., 2013b).

Consistent with our predictions, obese and healthy weight individuals exhibited opposing patterns of feeding-related functional connectivity between the dorsal mid-insula and the dorsal striatum. As a key component of striatal reward circuitry (Koob and Volkow, 2010), the dorsal striatum plays a central role in feeding. In rodent studies, disruption of dopamine signaling in the dorsal striatum alone is sufficient to abolish feeding (Sotak et al., 2005).

Between fasted and fed scans obese subjects exhibited a significant increase in connectivity between the mid-insula and the dorsal striatum. Importantly, the increase in functional connectivity between these regions varied in proportion to how appetizing the subjects rated the intervening meal. In contrast, healthy weight individuals exhibited a significant decrease in pre-to-post meal connectivity, in proportion to the meal pleasantness. Our observation that changes in dorsal striatum functional connectivity were correlated with meal pleasantness is in accordance with previous evidence linking this region to hedonic experience. In humans, feeding-induced reduction in D2 binding potential (indicative of local dopamine release) in the dorsal striatum is correlated with meal pleasantness in healthy non-obese subjects (Small et al., 2003). Furthermore, glucose metabolism in both the dorsal striatum and dorsal mid-insula covaries with the pleasantness of chocolate, as it is eaten to satiety (Small et al., 2001b).

Dopamine binding in positron emission tomography (PET) and fMRI BOLD signal in the dorsal striatum both exhibit abnormalities in obese humans. A recent PET study reported opposing relationships between dopamine binding and body-mass-index in the dorsal and ventral striatum, with dorsal striatum binding increasing as a function of BMI, and ventral striatum binding decreasing as a function of BMI (Guo et al., 2014). A shift in D2 receptor density from ventral to dorsal striatum is associated with the development of substance addiction (Volkow et al., 2013), consistent with models put forth that overeating in obesity may result from addiction to high-calorie food (Volkow et al., 2013). The divergent connectivity patterns observed between the dorsal striatum and the mid-insula in obese (compared to healthy weight) subjects may provide a link between the abnormal function of intrinsic reward circuitry in obesity, and the increased insula response to gustatory stimuli in obese individuals (Stice et al., 2008).

Within healthy weight subjects, a region of the orbitofrontal cortex (OFC; BA 11m/l) exhibited a positive relationship between change in mid-insula functional connectivity and change in hunger, pre-to-post meal. Therefore, as interoceptive self-reports of hunger decreased post-meal, so too did the connectivity between the dorsal mid-insula and the OFC. The OFC, in concert with the amygdala and mediodorsal thalamus, represents the moment-to-moment value of stimuli in the environment or behavioral outcomes, informed by the body's current state (Rudebeck and Murray, 2014). A similar region of the OFC has previously been identified as representing stimulus-reward associations for food rewards (Klein-Flugge et al., 2013), and the activity of this region of the OFC is likewise correlated with subjects' ratings of the inferred pleasantness of appetizing food pictures (Simmons et al., 2014). This region has been particularly implicated in selecting behavioral goals based upon satiety-related food value information, calculated in more caudal OFC regions (Murray et al., 2015). Thus, the observed relationship between hunger and mid-insula-OFC functional connectivity in healthy weight subjects may reflect one pathway by which visceral interoceptive information about hunger and satiety informs, or is informed by, the dynamic valuation of food stimuli. Importantly, inactivation of this region of the OFC in macaques results in the loss of the ability to adjust goal selection based upon updated satiety-related food value information (Murray et al., 2015). Thus the absence of this relationship between functional connectivity and hunger in the obese subjects might point toward a key aspect of the pathophysiology of obesity; namely, the inability to appropriately

modify goal-directed behavior toward food using homeostatically-relevant interoceptive information from the body. Conversely, as the activity of the dorsal mid-insula also strongly tracks food pleasantness (Small, 2010; Small et al., 2001a), the failure to observe this relationship in the obese subjects might reflect the inability of OFC satiety circuitry to properly regulate the insula's representation of food reward. Given the inherent limitations of resting-state functional connectivity analyses, we are unable to make conclusive claims about the directionality of this association.

In obese subjects, functional connectivity between the dorsal mid-insula and the striatum varied as a function of changes in hunger. While hungry, connectivity between the right dorsal mid-insula and the left ventral striatum/nucleus accumbens in obese subjects was decreased, in proportion to these subjects' subjective hunger ratings. The ventral striatum is one of the primary projection targets for dopaminergic neurons within the ventral tegmental area (Volkow et al., 2011). As a result, this area is a key region of the brain's reward system, and is heavily associated with incentive salience and motivational drive (Berridge et al., 2009), and is associated with encoding the hedonic value of sugar (Tellez et al., 2016). In short, our analyses demonstrated that the connectivity of homeostatically-sensitive insular cortex to a key ventral striatal region involved in food motivation varied with subjects' ratings of their experience of hunger. The qualitatively different functional connectivity patterns we observed between obese and healthy weight individuals as a function of hunger is consistent with one potential interpretation of our findings: whereas the healthy weight participants in this study may perceive hunger more as a physiological desire for energy, the obese subjects might perceive hunger more as an absence of reward. Combined with the abnormal dorsal striatum connectivity associated with meal pleasantness, this may signify a fundamental difference between obese and healthy weight individuals in the motivation behind eating, whereby food intake becomes less pleasurable and more compulsive (Volkow et al., 2013).

Limitations

Within this study, the sample of healthy comparison subjects was relatively smaller than obese participants (18 vs. 52), and was mainly composed of female participants, though the male/female proportion did not significantly differ between groups. Future studies, with a large and more gender-mixed healthy weight comparison group may be needed to verify the results of the present study. Additionally, we did not record any information on the menstrual cycle of our female participants in the present study, thus we are unable to control for any potential variations in reward processing across different phases of the menstrual cycle. This possibility will need to be explored in future studies.

Prior to post-meal scans, we provided subjects a 500-kcal meal that was standardized for energy and macronutrient content. In this respect, the total calorie and macronutrient consumption during this meal did not differ between subjects. However, because it would represent a smaller percentage of obese participants' daily caloric needs, the meal itself might not have been as filling for obese participants as it was for those with healthy weight. This is supported by the comparison of fullness ratings between participants (see Table II). However, a series of supplemental analyses identified that those differences in fullness

ratings did not appear to have a significant effect upon the results of our study (see Supplemental Materials p.2 and Table SIII for details). Though the possibility still remains that differences in gastric emptying and gastrointestinal transit between participant groups may have, in some fashion, contributed to the results of the present study. Future obesity studies where participants' diets and energy consumption can be more closely monitored may be able to account for these possibilities.

The subjects within this study fasted prior to their pre-meal scans for at least 4 hours, though the total amount of fasting may have varied somewhat among participants, as some participants that performed their fasted (pre-meal) scans prior to their fed (post-meal) scans had not eaten since the previous evening. However, follow-up analyses of order effects determined that alternating these scan orders had no effect upon the significant results of this study (see Table SII, Supplemental Materials section for details). Additionally, the resting-state scans examined within this study occurred at the end of scan sessions, after tasks involving food-cue presentation (which occurred during both pre-meal and post-meal scans), potentially affecting participants' resting connectivity following those scans. However, given that scan order was identical during both pre and post meal scans, any potential influence of food cue presentation prior to the resting scans is likely cancelled out.

Conclusion

Interoceptive signaling pathways help the brain's reward and decision circuitry to generate eating behaviors that are appropriate given the body's metabolic context, thereby promoting homeostasis in the short term, and healthy body weight in the long term. Previous work has demonstrated the sensitivity of the interoceptive insula to markers of energy signaling (Kroemer et al., 2013; Page et al., 2011; Simmons et al., 2013b; Wright et al., 2016), and that successful weight loss in young adults following behavioral interventions is associated with increased insula activity (Mata et al., 2016), as well as increased post-meal insula functional connectivity to the precuneus, a region that is a hub within the brain's default mode network (Lepping et al., 2015). Given these previous findings, as well as the findings of the present study, it seems likely that strategies that improve individuals' interoceptive sensitivity may promote eating behaviors that more closely track the body's energy needs. Indeed, a growing literature demonstrates that mindfulness-based interventions, which stress increased awareness and attention to interoceptive information from the body, do help individuals to make healthier decisions about what, and how much, to eat (Marchiori and Papies, 2014; Papies et al., 2015; Papies et al., 2016; O'Reilly et al., 2014; Daubenmier et al., 2016). The findings of the present study, and the growing recognition that interoceptive neurocircuitry makes important contributions to eating behavior, suggest that the field should continue to invest in developing new intervention approaches for obesity that help promote interoceptive sensitivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: Dorsal mid-insula seed region

The dorsal mid-insula seed region was functionally defined based on the results of prior published studies of gustatory and visceral sensory processing in different cohort of subjects. The seed region was a mask composed of the intersection of dorsal mid-insula voxels shown to be selective both for interoceptive attention to the viscera (Simmons et al., 2013a), as well as responsive to gustatory stimulation and food pictures (Simmons et al., 2013b).

Figure 2: Post-prandial connectivity between the Dorsal Mid-insula and Dorsal Striatum varies differentially between obese and healthy weight individuals, as a function of meal pleasantness. Obese participants exhibited increased functional connectivity pre-to-post meal between the right dorsal mid-insula and the right dorsal striatum. This increase in post-meal connectivity in obese participants was positively correlated to how appetizing they rated their meal. Healthy participants exhibited the exact opposite pattern, with decreased post-meal insulo-striatal connectivity that was negatively correlated to how appetizing they rated their meal.

Δ Hunger vs. Δ RSFC (Pre vs. Post Meal)

Figure 3: In obese individuals, post-prandial change in dorsal mid-insula connectivity is related to change in hunger

In obese and healthy weight participants, we observed regions where changes in mid-insula functional connectivity were related to changes in the subjects' subjective ratings of hunger. A) In obese participants, this included a region of the left ventral striatum, located in the head of the caudate, stretching back into the accumbens area. Specifically, those obese subjects who reported the greatest change in hunger post-prandially exhibited the greatest increase in functional connectivity between these regions involved in homeostatic processing and reward, respectively. B) Follow-up ROI analysis revealed that this relationship was present only in obese subjects, with healthy weight subjects exhibiting no relationship between changing hunger and changing connectivity between the dorsal mid-insula and the ventral striatum. C) Healthy weight participants exhibited a relationship between change in hunger and change in functional connectivity to a region of the left medial orbitofrontal cortex (OFC; BA 11m/11l). D) In these participants, hunger and medial OFC functional connectivity were positively related. As hunger ratings decreased post-meal, so too did connectivity between the medial OFC and the dorsal mid-insula.

Table I.

Participant demographics

	Obese	Healthy Weight	t_{68}	p
N	52	18		
Age	37.3 ± 8.0	35.1 ± 10.5	0.90	0.37
BMI	35.3 ± 3.6	21.5 ± 1.5	15.75	<0.001
Gender ¹	28F	14F	1.17 ¹	0.28
Education ²	4.7 ± 0.9	4.8 ± 1.1	4.77 ²	0.31
CESD	6.1 ± 4.8	4.6 ± 3.6	1.37	0.23
Appetizing	62.1 ± 20.9	63.7 ± 15.9	-0.29	0.77
Pre-meal Hunger	44.0 ± 25.1	46.1 ± 25.9	-0.3	0.76
Post-meal Hunger	7.1 ± 11.2	2.6 ± 2.8	1.7	0.10
Hunger Ratings	36.8 ± 24.3	43.5 ± 25.9	-0.98	0.33

¹Chi-square test of independence - $\chi^2(1)$

²Chi-square test of independence - $\chi^2(4)$

Center for Epidemiologic Studies Depression scale (CESD); Where appropriate, the table cells contain mean $\pm$ standard deviation.

Table II.

Pre and post-meal behavioral ratings

<i>Rating</i>	Group (OB vs. HW)		State (Pre vs. Post Meal)		Group $\times$ State	
	<i>F</i> (1, 136)	<i>p</i>	<i>F</i> (1, 136)	<i>p</i>	<i>F</i> (1, 136)	<i>p</i>
Hunger	0.08	0.78	148.40	< 0.001	0.79	0.38
Thirst	1.12	0.29	62.67	< 0.001	0.04	0.85
Fullness	0.10	0.76	101.25	< 0.001	3.55	0.06
Discomfort	0.02	0.90	0.68	0.74	0.11	0.74

OB – Obese Subjects; HW – Healthy Weight Subjects

Table III.

Brain regions exhibiting significant differences in mid-insula pre-post meal resting-state connectivity between obese and healthy weight participants.

Hemisphere / Location	Peak Coordinates ^a			Peak	Volume
	X	Y	Z	t ₆₈	(mm ³)
R Dorsal Striatum	+15	+7	+12	-4.0	392

^a All coordinates reported according to Talairach stereotaxic atlas.

Table IV.

Brain regions exhibiting a significant relationship between change in hunger ratings and pre-post meal change in resting-state functional connectivity to the right dorsal mid-insula.

Hemisphere / Location	Peak Coordinates ^a			Peak t	Volume (mm ³)
	X	Y	Z		
<i>Obese Subjects</i>				t(50)	
L Ventral Striatum / Accumbens area	-9	+11	-2	-4.4	368
<i>Healthy Weight Subjects</i>				t(16)	
L Medial Orbitofrontal Cortex	-11	+37	-16	5.7	408

^a All coordinates reported according to Talairach stereotaxic atlas.