Resting-state functional connectivity of brain regions involved in cognitive control, motivation, and reward is enhanced in obese females1–4

Mirjam A Lips, Marjolein A Wijngaarden, Jeroen van der Grond, Mark A van Buchem, Gerrit H de Groot, Serge ARB Romboots, Hanno Pijl, and Ilya M Veer

ABSTRACT

Background: The brain is an important regulator of both short- and long-term energy homeostasis. There is emerging evidence that food intake is under the control of cortical and subcortical areas involved in reward and cognition (1–4). Existence of an “obesogenic” pattern of brain activity—shaped by genetic, behavioral, and environmental factors—is suggested. It is hypothesized that chronic overstimulation by satiety and/or hedonic stimuli blunts the neuronal reaction to food intake, whereas consistent food restraint enhances the response to food (5). Therefore, the obesogenic pattern of brain activity is supposed to reflect a state of either insensitivity or hypersensitivity to food stimuli, depending on the nutritional state of the individual. To this extent, we examined the differences in brain functional connectivity (FC)5 between lean and obese [normal glucose tolerant (NGT) and type 2 diabetes mellitus (T2DM)] individuals after an overnight fast (the fasting condition) and directly after food intake (the satiated phase).

In the absence of food, the hypothalamus senses internal and environmental cues reflecting nutrient availability (2, 3). In the presence of satiety signals, however, hedonically driven corticolimbic regions, such as the orbitofrontal cortex, are also involved in the control of food intake (6). Furthermore, the reward network, of which the amygdala is a key constituent (1), responds differently to food-related stimuli in obesity. The amygdala is suggested to modulate the motivation for nonhomeostatic eating, and direct stimulation of amygdala neurons in rodents causes...
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Subjects and Methods

Subjects

The subjects’ characteristics are presented in Table 1. We included obese women who agreed to participate in a weight-loss trial comparing the effects of different weight-loss strategies (during the course of 2011). Fifty-six white obese women (of whom 26 were NGT and 30 had T2DM) with a BMI (in kg/m²; ±SD) of 42.8 ± 4.1 (range: 35–51) and aged 49.0 ± 6.1 y (range: 35–54 y) and 12 lean women (BMI: 21.7 ± 1.6, age: 49.2 ± 6.22 y) matched for age were included. Mean age and BMI were comparable between NGT and T2DM subjects.

Eighty percent of subjects were postmenopausal. Fasting glucose and glycated hemoglobin concentrations were greater in T2DM subjects than in NGT and lean subjects.

All subjects had been previously screened by a multidisciplinary team to check their eligibility to undergo bariatric surgery, according to international guidelines (16). Exclusion criteria were smoking, age >65 y, any chronic disease other than diabetes, psychiatric illness (including eating disorders), use of medication that could affect brain function (eg, antidepressants), and general MRI contraindications. For obvious reasons, we allowed our obese subjects to continue to use their antihypertensive medication (β-blockers, angiotensin-converting enzyme inhibitors) or statins. The subjects either were NGT or had T2DM according to WHO criteria (http://www.who.int/diabetes/currentpublications/en/). All diabetic subjects were treated with oral medication only (metformin and/or sulfonylurea derivatives). Subjects who reported the use of weight-loss medications within 90 d before enrollment of the study were excluded. The body weight of all subjects had been stable for ≥3 mo before inclusion. The participants were allowed to use cholesterol-lowering statins and antihypertensive medication. The control subjects were recruited via an advertisement and were healthy white women who were age matched (group level) to the obese subjects, had a BMI in between 20 and 25, and had a normal plasma glucose concentration in the fasting condition.

Because of excessive motion, large image artifacts, or incomplete scans, 10 scans of the obese subjects were excluded from further analysis. Therefore, the final sample comprised data from 46 obese subjects (n = 19 NGT and n = 27 T2DM). The study was performed in accordance with the principles of the revised Declaration of Helsinki. The protocol was approved by the medical ethics committee of the Leiden University Medical Center, and all subjects provided written informed consent before participation.

Study design

Subjects were studied after a 10-h overnight fast. All oral-glucose-lowering agents were discontinued 48 h before the study days. Anthropometric measurements (length, weight, and BMI) were made on arrival. The first MRI scan was made after the overnight fast. In between the fasting- and fed-state MRI scan sessions, the subjects ingested a 400-kcal liquid meal [266 mL Nutridrink (Nutricia, Danone); 49% carbohydrates (48.9 g dextrines; maltose and saccharose), 35% lipids (15.4 g), and 16% protein (15.9 g)] through a straw while remaining in a supine position in the MR scanner. Scanning was restarted after subjects finished their meal intake. Because the fMRI during the satiety state was performed between 10 and 25 min after meal ingestion, we studied the direct effects of food ingestion.

fMRI data acquisition

MRI scans were acquired with a Philips Achieva 3.0 Tesla scanner by using an 8-channel SENSE receiver head coil (Philips Health Care). Whole-brain resting-state scans were acquired by using T₂*-weighted gradient-echo echo-planar imaging [EPI; 160 volumes, 38 axial slices scanned in ascending order, repetition time (TR) 4400 ms, echo time (TE) 30 ms, flip angle 80°, field of view 220 × 220 mm, 2.75-mm isotropic voxels with a 0.25-mm slice gap]. A resting-state scan was performed in the
fMRI data preprocessing

FMRIB’s Software Library (version 4.1.3; www.fmrib.ox.ac.uk/fsl) (17, 18) was used to analyze the MRI data. First, preprocessing was performed on the resting-state scans by applying motion correction, brain extraction (to remove nonbrain data), spatial smoothing (Gaussian kernel of 6 mm full width at half maximum), a grand-mean intensity normalization of the entire data set by a single scaling factor, and a high-pass temporal filter with a cutoff of 0.01 Hz. The resting-state data set was registered to the high-resolution EPI scan, the high-resolution EPI scan to the T1-weighted anatomical image, and the T1-weighted anatomical image to the 2-mm isotropic Montreal Neurological Institute (MNI)-152 standard space (T1-weighted standard brain averaged over 152 subjects; MNI). Next, transformation matrices were concatenated to describe the registration of the resting-state data to MNI standard space, and the inverse matrix was calculated. Scans were excluded because of excessive motion (>3 mm translation or >3° rotation in any direction), large image artifacts, or incomplete scans (eg, due to claustrophobia during the scan).

fMRI time-course extraction and statistics

To study resting-state FC, a seed-based correlation approach was used with the hypothalamus, amygdala, and PCC as selected seeds. Binary spherical ROIs were created of the complete hypothalamus (bilateral) with a 2-mm radius (left seed: x = −4, y = −1, z = −13; right seed: x = 5, y = −1, z = −13), a 4-mm radius of the amygdala (left seed: x = −23, y = −4, z = −19; right seed: x = 23, y = −4, z = −19) (19), and a 4-mm radius of the PCC (seed: x = −5, y = −49, z = 40) (20). Our hypothalamus seed was based on the T1-weighted 1-mm isotropic standard space image of the MNI. We identified the middle voxel of the hypothalamus in both hemispheres and created a sphere around these. The 0 coordinate on the x axis was not the exact midline between the 2 hemispheres, but is slightly closer to the left hemisphere. Therefore, we had to move 1 mm to the right to arrive at a midpoint of the right hypothalamus comparable with that of the left. These ROIs were registered to each participant’s preprocessed resting-state data set by using the inverse transformation matrix.

The mean time course within the left and right seeds of each ROI (except for the PCC, only comprising one medial seed) was calculated and used as a regressor in a general linear model for each of the networks. In addition, white matter signal, cerebrospinal fluid signal, 6 motion parameters (3 translations and 3 rotations), and the global signal were used as nuisance repressors. For each individual, the 3 general linear model were analyzed by using the FMRI Expert Analysis Tool [version 5.98, part of FMRIB’s Software Library (17)]. Contrasts were made for the left and right seeds together. The resulting parameter estimate maps were then resliced into 2-mm MNI space and analyzed into a higher level mixed-effects model to assess between-groups effects in the fasting state and within- and between-group difference between the fasting and the fed states. No between-group effects were found between NGT and T2DM obese subjects as tested by post hoc tests. As a consequence, between-group effects were compared between lean and obese (NGT and T2DM together) subjects by using independent-samples t tests. Whole-brain z-statistical images were thresholded with an initial cluster-forming threshold of z > 2.3 and a corrected cluster significance threshold of P < 0.017 (ie, P < 0.05 Bonferroni corrected for the 3 networks of interest: hypothalamus, amygdala, and PCC) (21). Masks were created from the significant and adjacent voxels, for which we found differences in the FC analyses. Next, the average z scores were extracted from these masks for each individual and used to plot the significant whole brain–corrected connectivity differences.

RESULTS

General functional connectivity

The main effects for FC of the hypothalamus, amygdala, and PCC are described elsewhere (see Supplementary text and supplementary Figures 1, 2, and 3 under “Supplemental data” in the online issue). The timeline of the study protocol is also depicted elsewhere (see Supplementary Figure 4 under “Supplemental data” in the online issue).

Connectivity in obese NGT and obese T2DM subjects

We were primarily interested in defining differences between lean and obese subjects. However, because we included obese subjects who were NGT and obese subjects with T2DM in our study, we checked for differences between obese NGT subjects and obese T2DM subjects. We found no differences between NGT and T2DM obese subjects in the fasting state or in response to food intake (see Supplementary Tables 1 and 2 under “Supplemental data” in the online issue); therefore, we decided to analyze the differences between lean and obese, thereby combining obese subjects with NGT and T2DM obese groups.

Functional connectivity with the hypothalamus

After an overnight fast

In the fasting state, FC between the hypothalamus and 2 areas in the medial prefrontal cortex (PFC), the frontal pole, and dorsal anterior cingulate cortex was stronger in obese subjects than in lean subjects (Table 2, Figure 1). In addition, lean subjects had stronger negative connectivity between the hypothalamus and the bilateral inferior frontal gyrus (IFG), which was greatly decreased in obese subjects. Last, obese subjects showed positive connectivity between the hypothalamus and the bilateral caudate nucleus, putamen, and insula, whereas negative connectivity with these areas was found in the lean group.
TABLE 2
Hypothalamic functional connectivity in the fasting condition: differences between the lean and obese groups, and the effect of meal intake on connectivity between groups

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Region</th>
<th>Peak voxel MNI(^2) coordinates (x/y/z)</th>
<th>Group contrast</th>
<th>(z) score(^2)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting condition</td>
<td>Frontal pole</td>
<td>0/58/4</td>
<td>Between group obese &gt; lean</td>
<td>3.76</td>
<td>0.0002</td>
</tr>
<tr>
<td>Right</td>
<td>Dorsal anterior cingulate cortex</td>
<td>8/40/20</td>
<td>Between group obese &gt; lean</td>
<td>4.04</td>
<td>0.0001</td>
</tr>
<tr>
<td>Left</td>
<td>Dorsal anterior cingulate cortex</td>
<td>-10/40/14</td>
<td>Between group obese &gt; lean</td>
<td>3.73</td>
<td>0.0002</td>
</tr>
<tr>
<td>Right</td>
<td>Inferior frontal gyrus</td>
<td>48/34/12</td>
<td>Between group obese &gt; lean</td>
<td>3.67</td>
<td>0.0002</td>
</tr>
<tr>
<td>Left</td>
<td>Inferior frontal gyrus</td>
<td>-50/10/18</td>
<td>Between group obese &gt; lean</td>
<td>4.09</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Right</td>
<td>Caudate</td>
<td>16/18/14</td>
<td>Between group obese &gt; lean</td>
<td>3.45</td>
<td>0.0006</td>
</tr>
<tr>
<td>Left</td>
<td>Caudate</td>
<td>-18/20/10</td>
<td>Between group obese &gt; lean</td>
<td>3.53</td>
<td>0.0004</td>
</tr>
<tr>
<td>Right</td>
<td>Putamen</td>
<td>22/10/10</td>
<td>Between group obese &gt; lean</td>
<td>2.99</td>
<td>0.0028</td>
</tr>
<tr>
<td>Left</td>
<td>Putamen</td>
<td>-26/12/8</td>
<td>Between group obese &gt; lean</td>
<td>3.12</td>
<td>0.0018</td>
</tr>
<tr>
<td>Right</td>
<td>Insula</td>
<td>32/12/12</td>
<td>Between group obese &gt; lean</td>
<td>2.77</td>
<td>0.0056</td>
</tr>
<tr>
<td>Left</td>
<td>Insula</td>
<td>-36/4/8</td>
<td>Between group obese &gt; lean</td>
<td>2.57</td>
<td>0.0102</td>
</tr>
<tr>
<td>Effect of meal intake</td>
<td>Left Inferior frontal gyrus</td>
<td>-50/20/8</td>
<td>Between group obese &gt; lean</td>
<td>3.48</td>
<td>0.0005</td>
</tr>
<tr>
<td>Left</td>
<td>Insula</td>
<td>-40/2/8</td>
<td>Between group obese &gt; lean</td>
<td>2.43</td>
<td>0.015</td>
</tr>
</tbody>
</table>

\(^1\)MNI, Montreal Neurological Institute.
\(^2\)All \(z\) values were cluster corrected for multiple comparisons (\(z > 2.3, P < 0.017\)).

After food intake

In the left IFG and insula, a group \(\times\) meal interaction was found: in lean subjects, the negative connectivity with the hypothalamus in the fasting state diminished on food intake, comparable with that in the obese subjects after an overnight fast, whereas hypothalamic connectivity in the obese group was not affected by food intake (Table 2, Figure 1). Of note, whereas only the left IFG and left insula showed the corrected interaction effect, the right IFG, right insula, bilateral caudate nucleus, and medial PFC showed a trend for this interaction (\(P < 0.001\), uncorrected; data not shown). In lean subjects only, additional regions showed an effect of meal intake (see Supplementary Table 3 under “Supplemental data” in the online issue): the left frontal pole and the putamen. In these regions, hypothalamic connectivity switched from negative to positive.

Functional connectivity with the amygdala

After an overnight fast

In the fasting state, 2 regions showed a difference between the 2 groups: the amygdala was positively connected with the right insula in obese subjects, whereas negative connectivity was observed in lean subjects (Table 3, Figure 2). Lean subjects showed strong negative connectivity between the amygdala and the right IFG, whereas the obese subjects barely showed any connectivity between the amygdala and the IFG.

After food intake

No group \(\times\) meal interaction was observed for the amygdala.

Functional connectivity with the PCC

No differences in FC of the PCC were observed between the 2 groups, either in the fasting state or after meal intake (no group \(\times\) meal interaction).

Association between functional connectivity and metabolism

The changes in FC in response to food intake were correlated with the metabolic variables, such as glucose and insulin concentrations. However, we did not observe any significant correlations between fasting/postprandial glucose and insulin concentrations and FC in our subjects.

DISCUSSION

We compared the FC of the hypothalamus and amygdala, probing FC networks involved in the homeostatic control of food intake and body weight, in the fasting state and in response to food intake in lean and obese participants. We also studied connectivity of the PCC, probing the default mode network, which was associated with appetite and obesity in previous studies (15, 22). In general, the architecture of the hypothalamus, amygdala and PCC FC networks reported here corresponds well with what is known about the outline of the associated networks in the literature (see supplementary figures under “Supplemental data” in the online issue) (20, 23–26).

Perhaps surprisingly, given the known differences between the T2DM and insulin-sensitive (NGT) subjects (27), we found no differences between the NGT and T2DM obese subjects in the fasting state or in response to food intake. One possible explanation for this was the degree of insulin resistance in our NGT obese subjects, which is known to (at least in part) influence FC levels and, as such, could have affected possible differences between the T2DM and obese insulin-sensitive subjects. Indeed, defective insulin signaling in the brain has been described in obese subjects without diabetes (28). Moreover, rodent studies have shown that even short periods of high-fat feeding induce a certain inflammatory state in the brain characterized by reactive gliosis, before substantial obesity has been reached. Also in obese humans, evidence was found for increased gliosis in the
hypothalamus (29). These data, at least in part, explain why altered FC was present in our NGT and T2DM subjects as compared with our lean subjects.

During the “food-wanting” state we observed differences in hypothalamic connectivity with the PFC, which is involved in a central orexigenic network (30), the insular cortex and dorsal striatum. The PFC has an established role in cognitive and emotional control and has been suggested to induce the termination of feeding and the ability to predict the satisfactory effects of food intake (31, 32). Enhanced positive hypothalamic connectivity with the PFC in obese subjects after an overnight fast might be involved in an increased craving for food during the temporary absence of food.

In addition, we found that connectivity between the amygdala and right IFG/insula was stronger in lean than in obese subjects. The amygdala is involved in the response to motivationally

FIGURE 1. Between-group differences in hypothalamus functional connectivity with the medial prefrontal cortex, bilateral inferior frontal gyrus, and caudate nucleus in the fasting condition (A) and with the left inferior frontal gyrus and insula in response to meal intake (B). Results are projected on the 2-mm standard space template of the Montreal Neurological Institute. Between-group effects are thresholded at $z > 2.3, P < 0.017$ (cluster corrected). The left side of the brain corresponds to the right side of the picture and vice versa (radiological convention). Bar graph values are depicted as mean ($\pm$SD) $z$ scores. Pre, fasting condition; Post, after meal intake.
relevant cues, such as hunger, and plays an important role in food reward (1). As mentioned before, the insula connects perception of internal stimuli to aspects of emotion and motivation (33). Although speculative, connectivity between the amygdala and insula in lean subjects after an overnight fast could reflect a perception of the food-deprived state, whereas obese people do not perceive this state accordingly.

In response to food intake, we mainly observed changes in lean subjects, whereas connectivity in obese subjects barely changed. Interestingly, in lean subjects, hypothalamus FC changed on food intake, to become similar to the FC observed in obese subjects before and after food intake. Specifically, the negative hypothalamic connectivity with the left inferior frontal cortex and insula found before the meal decreased in lean subjects, whereas no effect was observed in obese subjects. In addition, within the lean subjects, connectivity with the left frontal pole and putamen even tended to switch from negative to positive. The insula is involved in consciousness and self-perception and connects the perception of internal stimuli to aspects of emotion and motivation (33). The hypothalamus and insula are suggested to be part of the so-called salience network (34) that perceives internal and external cues to adapt behavior and/or physiology accordingly (35). We propose that our current results—strong hypothalamic-insula connectivity in lean individuals in the food-wanting phase and a reduction after food intake—may reflect an ability to “evaluate” the perception of hunger in the fasting state in lean subjects and a “satiation” of this signal after food intake, whereas in obesity there is a certain insensitivity to these food-related cues. A comparable effect of diminishing connectivity with the frontal pole after food intake was observed in lean subjects, again more resembling the obese pattern of connectivity before and after food intake. However, this was not significantly different between groups. Although speculative, this trend may again suggest an insensitivity of food-regulatory cues in obese subjects, whereas in lean subjects there is effective dampening of the food-wanting signal.

Hypothalamic connectivity with the dorsal striatum (caudate and putamen) was enhanced in obese subjects as compared with that in lean subjects, and only in lean subjects was connectivity affected (became positive) after food intake (see Supplementary results under “Supplemental data” in the online issue). Dopaminergic circuits in the dorsal striatum are known to be involved in food reward (36), and caudate activity has been shown to be enhanced in obese subjects in the hyperinsulinemic state (37). Conversely, it has been suggested that relative or absolute dopamine deficiency in obese subjects perpetuates pathological

<table>
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<th>Hemisphere</th>
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<th>Peak voxel MNI (^1) coordinates (x/y/z)</th>
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<th>(z) score (^2)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>Insula</td>
<td>36/-22/18</td>
<td>Between group</td>
<td>3.51</td>
<td>0.0004</td>
</tr>
<tr>
<td>Right</td>
<td>Inferior frontal gyrus</td>
<td>48/10/24</td>
<td>Between group</td>
<td>4.23</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^1\)MNI, Montreal Neurological Institute.

\(^2\)All \(z\) values were cluster corrected for multiple comparisons (\(z > 2.3, P < 0.017\)).
eating as a means to compensate for decreased activation of these circuits (38, 39). As such, the lack of hypothalamic-striatum connectivity in the hyperinsulinemic postprandial state in obese subjects conceivably blunts food-related reward sensations.

Of interest, recent data suggest that massive weight loss induced by bariatric surgery is able to (at least in part) restore proper hypothalamic function and reduce the mentioned inflammatory state induced by obesity (40). Further research will have to provide the answers to whether these changes in hypothalamic function are reversible.

We found differential FC of brain areas to be implicated in motivation and reward in the fasting state in obese participants but not in lean subjects, which appeared to be unaffected by food intake. It has been suggested that obesity is associated with an imbalance between brain circuits promoting reward seeking and those governing cognitive control. Obese subjects typically show enhanced responsiveness to external food cues, such as visual and olfactory stimuli (8, 41, 42). This response may involve abnormal stimulus response learning and incentive motivation governed by the caudate nucleus, due to abnormally high inputs from the amygdala and insula, and dysfunctional inhibitory control by prefrontal regions (37). Our data add to this hypothesis because we showed enhanced FC strength of prefrontal regions in the fasting state, whereas eventual food intake did not elicit any changes in connectivity between reward areas in the obese subjects, apparently rendering the brain unable to differentiate between hunger and satiety.

Several limitations of the current study need to be addressed. First, only female subjects were included because of the low response rate of male subjects willing to participate. Given the known differences in metabolic and neuronal function between males and females (43), we decided to include female subjects only to avoid a sex bias in our study. As a consequence, our results can only be generalized to females, whether the effects found also pertain to the male obese population has yet to be established. Eighty percent of our population was postmenopausal, and the remaining 20% were not scanned in a particular phase of their menstrual cycle. This may have affected our results slightly. Second, we were aware of the discrepancy between the numbers of subjects included in the lean and obese groups. To define whether this could have biased our results, we performed a post hoc analysis to test homogeneity of variance, which showed a normal distribution of z scores and homogeneity of variance in both groups. Third, we used a mixed meal, instead of glucose only, to evoke a physiologic stimulus. This was a restriction, because different brain signaling is evoked depending on the type of macronutrients (44). Fourth, we were unable to find any significant correlations between physiological variables (eg, HOMA-IR) and connectivity. Moreover, because of the complexity of our current study protocol, we were unable to record any behavioral and subjective measurements. In future studies, it would be interesting to relate measures of subjective hunger and satiety to FC data.

Hypothalamic and amygdala FC may, in addition to changes in homeostasis, be influenced by noise from surrounding arteries (45). Because we did not register respiration and heart rate, we used the global signal in our analysis as a nuisance regressor, which previously showed strong correlations with physiologic noise sources in fMRI data (46). Because global signal regression could mathematically induce negative correlations or even introduce between-group differences (47, 48), no strict conclusions can be drawn from the observed sign (eg, inhibition or stimulation) of the connectivity. However, global regression increases the signal to noise and connectivity specificity and, as such, differences in connectivity are real. In addition, the regions found in our analysis are known to be important in the regulation of food intake and obesity. Nevertheless, replication of the current results with better monitoring of physiological variables is warranted.

Last, because of the relatively low spatial resolution of the resting-state data acquired, it was impossible to make distinctions between the different hypothalamic regions, which is unfortunate because different hypothalamic neurons and nuclei have distinct homeostatic properties.

In conclusion, our results add to the existing evidence that FC in several brain networks, in particular the central orexigenic network and the reward network, is altered in obese subjects—most strikingly in the fasting state. Obesity is associated with distinct FC of brain areas implicated in cognitive control, motivation, and reward in the fasting state, whereas food intake has limited effects on these connections compared with those in lean subjects. This finding is in line with the hypothesis that obesity is associated with an imbalance in brain circuits promoting reward seeking and governing cognitive control.

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