Visceral Adiposity and Insular networks:
Associations with Food Craving

Oren Contreras-Rodríguez 1,2*, Cano M 1,2,3, Raquel Vilar-López 4,5,6, Jacqueline Schmidt Rio-Valle1, Juan Verdejo-Román4, Juan F Navas 4,8, Cristina Martín-Pérez 7,6, Fernando Fernández-Aranda 1,9, José Manuel Menchón1,2, Carles Soriano-Mas 1,2,10*, Antonio Verdejo-García 6,11

1. Psychiatry Department, Bellvitge University Hospital, Bellvitge Biomedical Research Institute-IDIBELL, Barcelona Spain
2. Ciber en Salud Mental (CIBERSAM-17), Instituto Salud Carlos III, Barcelona, Spain
3. Department of Clinical Sciences, School of Medicine, University of Barcelona, Spain
4. Mind, Brain and Behavior Research Center, University of Granada, Spain
5. Departamento de Personalidad, Evaluación y Tratamiento Psicológico
6. Red de Trastornos Adictivos, University of Granada, Spain
7. Department of Nursing, School of Health Sciences. Universidad de Granada, Spain.
8. Department of Experimental Psychology, University of Granada, Spain
9. Ciber en Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto Salud Carlos III, Barcelona, Spain
10. Department of Psychobiology and Methodology of Health Sciences, Autonomous University of Barcelona, Spain.
11. School of Psychological Sciences and Monash Institute of Cognitive and Clinical Neurosciences, Monash University, Melbourne, Australia

Number of words: Abstract 269; Text body 3080

The submission contains: The main Text file, 3 Figures, 2 Tables, 1 Supplementary Information file

*Address correspondence to Carles Soriano-Mas and Oren Contreras-Rodríguez.
Psychiatry Department, Bellvitge University Hospital, Bellvitge Biomedical Research Institute-IDIBELL, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona.
Tel: (+34) 93 2607500 (ext.2864) Fax: (+34)932607658
Email: csoriano@idibell.cat, ocontreras@idibell.cat

Conflict of Interest Statement: The authors declare no conflict of interest. This study has been funded by the Project Grant NEUROCOBE (PI-HUM-6635) from the Andalusian Council of Innovation, Science and Industry to Dr. Soriano-Mas is funded by a ‘Miguel Servet’ contract (CPI16/00048) and Dr. Oren Contreras-Rodriguez is funded by a ‘Sara Borrell’ postdoctoral fellowship (CD14/00246) from the Carlos III Health Institute (ISICII). Juan Francisco Navas is funded by a FPU predoctoral fellowship (FPU13/00669) from the Spanish Ministry of Education, Culture and Sport. CIBERSAM and CIBEROBN are both initiatives of ISCIII.
Abstract

**Background/Objectives.** Accumulation of visceral adiposity can disrupt the brain’s sensitivity to interoceptive feedback, which is coded in the insula. This study aimed to test the link between visceral fat and the functional connectivity of two insulae regions relevant for eating behavior: the middle-dorsal insula (mIns), which codes homeostatic changes, and the rostral insula (rIns), which codes stable representations of food properties. We also assessed the impact of visceral adiposity associated insulae networks on food craving. **Subjects/Methods.** Seventy-five adults ranging in weight status (normal and excess weight) underwent resting-state functional magnetic resonance imaging (fMRI) and subjective food craving measures. We examined the association between visceral fat and seed-based functional connectivity of the mIns and the rIns, controlling for BMI, age and sex, using multiple regressions in SPM8. We also tested if visceral fat mediated the association between insulae connectivity and food craving. **Results.** Higher visceral adiposity was associated with decreased connectivity between the mIns and a cluster involving the hypothalamus and the bed nucleus of the stria terminalis. Decreased connectivity in this network was associated with greater food craving, a relation mediated by visceral adiposity. Visceral adiposity was also associated with increased connectivity between the mIns and the middle frontal gyri and the right intraparietal cortex, and between the rIns and the right amygdala. **Conclusions.** Accumulation of visceral adiposity is linked to disrupted functional connectivity within the mIns and rIns networks. Furthermore, the link between the mIns network and food craving is mediated by visceral fat. Findings suggest that visceral fat disrupts insula coding of bodily homeostatic signals, which may boost externally driven food cravings.
**Introduction**

Excessive adipose tissue is associated with greater risk of diabetes, metabolic syndromes, cardiovascular disease, dementia, and cancer (1). Accumulation of visceral (versus subcutaneous) adiposity has been specifically associated with brain harms (2), probably via hyper-secretion of adipocytokines and activation of neuroinflammatory pathways (3). An intriguing possibility, which has not been previously tested, is that visceral adiposity accumulation disrupts the brain’s sensitivity to the interoceptive signals that normally guide homeostatic eating. In fact, greater visceral adiposity has been associated with dysregulated eating behaviors, such as addictive-like eating (4–6).

If that were the case, visceral fat accumulation would be associated with functional alterations of the insula, which is the key brain region for perception of internal bodily signals (7).

Neuroimaging studies have consistently found associations between visceral adiposity levels and both structural alterations (8–10) and increased activations to caloric foods (11,12) in the anterior and mid-posterior insular regions. Posterior sections of the insula are primarily involved in processing interoceptive signals (7,13), therefore changing its activation in relation to the homeostatic states of hunger and satiety (14,15).

Conversely, anterior insular sections steadily hold stable representations of external-cues (14), thanks to connections with other regions of the salience network, such as the dorsal anterior cingulate and the orbital-opercular cortices, as well as with limbic structures such as the amygdala (16). Importantly, visceral fat may disrupt posterior insula networks processing of interoceptive bodily inputs and sensitize anterior insula networks processing to external food-cues (17,18). These alterations may explain why food-related decisions in people with obesity tend to rely on external food cues rather
than on bodily feedback. This tendency may be especially relevant in Western societies, given wide availability of highly palatable unhealthy food (19,20).

In this context, a comprehensive assessment of the different (i.e., interoceptive vs. externally oriented) insular networks in relation to visceral adiposity may help us to better understand the modulation of eating behavior regulatory brain circuits by peripheral signals. Specifically, this study aimed to examine whether visceral adiposity has an impact on the functional connectivity of posterior (middle dorsal, mIns) and anterior (rostral, rIns) insulae regions (14). Likewise, we also investigated whether the putative association between visceral adiposity-and insulae functional networks was related to heightened food craving. We hypothesized that greater levels of body visceral adiposity will modulate the connectivity between the mIns and interoceptive processing regions, such as the hypothalamus (17,18), and between the rIns and saliency processing regions, such as the amygdala, dorsal anterior cingulate cortex, and orbital-opercular cortices (11,12,16). Moreover, we expected that such associations between functional connectivity patterns of the insula and visceral adiposity was also related with increased food craving (11,12,18).


Materials/Subjects and Methods

Subjects

Seventy-five adults with a range of visceral fat levels participated in the study [men
n=34(45.3%) and women n=41(54.6%)]. In typical scenarios, a sample size of n= 75 is
enough to obtaining stable correlation estimates in neuroimaging studies. Participants
were recruited via general hospitals and community advertisements (i.e., local press,
radio and social media), and enrolled if they were aged 18 to 45 and had a Body Mass
Index (BMI) >18. Exclusion criteria were: (i) presence of current or past medical illness
(e.g., metabolic disease) or psychiatric disorders (e.g., eating or emotional disorders), as
evidenced by semi-structured interviews, (ii) MRI contraindications (e.g.,
claustrophobia, ferromagnetic implants), (iii) self-reported use of psychotropic
medication, (iv) morbid obesity (BMI≥40), and (v) the presence of food allergies to the
ingredients used during a tasting session. The “Universidad de Granada Human
Research Ethics Committee” approved the study, and all participants provided informed
consent.

Measures

Imaging data Acquisition

All participants were scanned at the same time of the day (4-6 pm), after lunch, which in
Spain is typically between 2 and 4 pm, and ratings of hunger (0-100 VAS) indicated
that participants were satiated (not hungry, not full) both at the beginning and at the end
of the scanning session (Table 1). Participants underwent a 6-minute resting-state scan.
They were instructed to lie still with eyes closed. We used a 3.0 Tesla clinical MRI
scanner, equipped with an eight-channel phased-array head coil (Intera Achieva Philips
Medical Systems, Eindhoven, The Netherlands). A T2*-weighted echo-planar imaging
(EPI) was obtained (TR=2000 ms, TE=35 ms, FOV=230 x 230 mm, 96x96 pixel matrix; flip angle=90º, 21 4-mm axial slices, 1-mm gap, 180 whole-brain volumes). The sequence included four initial dummy volumes to allow the magnetization to reach equilibrium.

Visceral adipose tissue

Visceral adiposity was measured using a body composition analyzer TANITA BC-420 (GP Supplies Ltd., London, UK), which estimates the adiposity that accumulates in the intra-abdominal cavity and around the organs through bioelectric impedance. This measure ranges from 0-60, with values between 1-12 indicating normal levels of visceral adiposity, and values between 13-59 indicating excessive visceral adiposity, and we used these scores as a dimensional measure of visceral adiposity accumulation. This method has shown to provide reliable measures of visceral adiposity, yielding higher correlations with MRI, which is the gold standard measure of visceral adiposity (r=0.89 and r=0.84, in women and men respectively), than those found between MRI measurements and waist circumference (r=0.79, r=0.79) or BMI (r=0.64, r=0.55) (21).

Food craving assessment

Participants were shown nine photographs of highly appetizing food stimuli, i.e., rich in sugar and fat content (e.g., cheesecake, chocolate) and healthier foods, i.e., low sugar and fat, immediately after the imaging assessment. These images were professional photographs of the foods tasted during the catered session, standardized in terms of light and complexity. They were instructed to rate their level of desire to consume them using a visual analogue scale (VAS, range 0– 10). To maximize sensitivity of the analyses, food craving was computed by subtracting the desire to consume plain vs. appetizing
foods; therefore, positive values reflected higher craving (i.e., increased desire) to
to consume appetizing foods. Moreover, to maximize tasks’ validity, all participants were
pre-exposed to these foods in a catered tasting session conducted 1 week before the
assessment. The participants were invited to participate in the catered tasting session
right after giving the consent to participate in the study. In the catered session the
participants were invited to taste and rate the liking of the foods (VAS, range 0-10) that
will be subsequently presented to them again in the imaging assessment.

Analyses

Behavioral

Sociodemographic and behavioral data were analyzed using IBM SPSS 21.0.0 (IBM
Corp., Armonk, NY). The level of significance was set at p<0.05.

Brain Imaging

Preprocessing. The functional imaging data was processed and analyzed using
MATLAB version R2008b (The MathWorks Inc, Natick, Mass) and Statistical
Parametric software (SPM8; The Welcome Department of Imaging Neuroscience,
London). Preprocessing steps involved motion correction, spatial normalization and
smoothing using a Gaussian filter (FWHM 8 mm). Data were normalized to the
standard SPM-EPI template and resliced to a 2mm isotropic resolution in Montreal
Neurological Institute (MNI) space.

Insula seed-based functional connectivity analyses. Insulae subregions were identified
in each hemisphere. Following prior work on dissociable aspects of the insula that are
relevant to food processing (14), four seeds of interest were placed bilaterally in dorsal mid-insulae (mIns, \(x=\pm 33, y=5, z=14\)) and rostral insulae (rIns, \(x=\pm 35, y=11, z=2\)) using 2-mm-radius spheres. The mIns seeds were located near the fundus of the superior circular insular sulcus at the intersection of the insula and the overlying operculum. The rIns seeds were located along the anterior short insular gyrus, rostral and inferior to the dorsal mid-insula seeds. Importantly, these seeds were spatially separated by more than 8mm (>1 FWHM).

A first-level (single subject) t-test maps was estimated that included the bilateral mean activity time courses (extracted using the Marsbar toolbox) (22) for the mIns and rIns seeds, together with nuisance signals, as predictors of no interest in whole-brain SPM8 linear regression analyses. Nuisance signals included six head-motion parameters (3 translations and 3 rotations) and the time courses representing mean signal fluctuations in white matter, cerebrospinal fluid and the entire brain. A high-pass filter (128-seconds) was used to remove low-frequency drifts. Two contrast images representing the bilateral mean connectivity of the mIns and rIns seeds were generated for each participant in first-level (single-subject) model, and these were included in separate second-level multiple regression models (one per seed), with the index of visceral fat as the unique predictor of interest to explore for whole-brain insula functional connectivity-visceral fat associations. BMI, sex and age were entered as nuisance variables in these analyses. Significance thresholds were determined by 1000 Monte Carlo simulations using AlphaSim as implemented in the SPM RESTplus V1.2 (Resting-State fMRI Data Analysis Toolkit) toolbox (23). The input parameters to AlphaSim included an individual voxel threshold probability of \(P<0.001\), a cluster connection radius of 5 mm, and the actual smoothness of imaging data after model
estimation, incorporating a binarized grey matter mask of 128,190 voxels (2x2x2mm).

Cluster estimation was 880 mm$^3$ (101 voxels).

Correlation and mediation analysis with food craving

We performed correlation analyses between food craving scores and the beta values from the peak coordinates of those brain regions showing a significant association with visceral adiposity in the preceding multiple regression analyses. Signal extraction from the peak coordinates was performed by non-adjusting for the variables entered in the multiple regression model (i.e., visceral adiposity, BMI, age, sex), and therefore beta values represent the strength of the functional connectivity between the seeds (mIns or rIns) and the voxels showing a significant association with visceral adiposity. Likewise, correlations between food craving and visceral adiposity were also explored. All these analyses were performed by assessing Pearson two-tailed correlations in SPSS.

To complement the above analyses, we also tested whether the relationships between insular connectivity (X) and food craving (Y) were mediated by the index of visceral adiposity (M) using IBM SPSS Amos 21.0.0. Specifically, we tested the working hypothesis that visceral adiposity modulates the brain pathways involved in interoception and food craving (6). These mediation analyses first tested: whether insular functional connectivity predicted visceral adiposity (path a); whether visceral adiposity predicted food craving, controlling for insular connectivity (path b); and whether insular functional connectivity predicted food craving (path c). Next, this model assessed whether visceral adiposity explained a significant proportion of the covariation between the insular functional connectivity and food craving (path c’) and the mediation effect (a*b effect) with bootstrapping methodology (5000 bootstrap samples) (24). The model was tested for main fit indices, including the goodness of fit index (GFI), the root mean square error of the approximation (RMSEA), and the
comparative fit index (CFI). Considering Kolmorov-Smirnov normality tests and the large number of subjects included in our study, we used parametric statistical analyses in all the above described analyses.
Results

Behavioral data

Participants (n=75) showed a mean level of visceral adiposity of 5.61, ranging from 1 to 19. For all but two cases the indices of body visceral adiposity levels fell below the range indicating excessive levels of visceral adiposity. Women and men did not differ in the index of visceral adiposity (women= 4.39, men = 7.08, chi-square test p= 0.199).

Visceral adiposity was strongly associated with BMI (r= 0.854, p= 0.001) and showed a tendency towards a positive association with age (r= 0.210, p= 0.071), but no significant associations were found with the reported levels of hunger before (r= 0.117, p= 0.324) and after (r= 0.090, p= 0.455) the scanning session. Furthermore, participants reported a subjective higher desire for the consumption of appetizing relative to standard foods (mean ± SD; appetizing= 5.62 ± 1.39, standard= 5 ± 1.22, p= 0.001). Food craving measurements were not associated with the reported levels of hunger before (r= 0.078, p= 0.512) and after (r= 0.084, p= 0.486) the scanning session.

Correlations between Insulae connectivity and Visceral Fat

The functional connectivity maps of the rIns and mIns seeds yielded segregated networks, with the mIns mainly connected with subcortical regions, and the rIns showing greater connectivity with cortical regions (positive connectivity with parts of the salience network and other sensory processing cortices, and negative connectivity with interoceptive and default mode processing regions). See Table S1 and Figure S1 for further details about the specific brain functional connectivity within the rIns and mIns networks. The results from additional two-sample t-test analyses examining sex and hemispheric interactions in the functional connectivity of the insulae seeds are reported in the Supplementary Material. Significant correlations between visceral adiposity and insulae connectivity networks are reported below.
Dorsal-mid insula (mIns): Higher levels of visceral adiposity were associated with a decreased functional connectivity between mIns and a cluster encompassing the hypothalamus (HYP, lateral hypothalamus and preoptic nuclei) and the bed nucleus of the stria terminalis (BNST), which also extended to the right ventral striatum. Likewise, visceral adiposity levels were associated with an increased functional connectivity between the mIns and the bilateral middle frontal gyri, as well as the right intraparietal gyrus (Figure 1, Table 2).

Rostral Insula (rIns): Visceral adiposity levels did not show significant associations with rIns connectivity at the selected whole-brain level of significance (P<0.001, 101 voxels). Nevertheless, to investigate our hypothesis that visceral adiposity would be associated with an increased functional connectivity between the rIns and brain regions within the salience network, we explored the functional connectivity of the rIns within a mask encompassing regions of the salience network (16) that have shown a significant association with visceral adiposity measures in a sample of 2344 adults (9). The orbital-opercular cortices, the amygdala, and the dorsal anterior cingulate cortex (Brodmann area 24) met these criteria and were therefore defined with the WFU PickAtlas software 3.0.4 (25) (see Figure S2). In this analysis, we used small-volume correction procedures to correct the significance threshold, which was set at P_{FWE}<0.05 within the region of interest. Using this approach, higher levels of visceral adiposity were associated with an increased functional connectivity between the rIns and the right amygdala (Figure 1, Table 2).

Correlation and mediation analyses with food craving
Food craving showed a specific negative association with the functional connectivity between the mIns and the BNST/HYP (r = -0.24, p = 0.035, Figure 2a). No significant associations were found with the other brain regions reported in the above analyses. A significant positive association between food craving and body levels of visceral adiposity (r = 0.31, p = 0.006) was also observed (Figure 2b). The mediation model showed that the negative association between the functional connectivity of the mIns-BNST/HYP network and food craving (path c), was fully mediated by visceral adiposity (path c'). The mediation effect was significant at p = 0.002. The standardized regression weights for each path are shown in Figure 3. This model showed good fit statistics (chi-square, $\chi^2 = 6.620$, df = 7, p = 0.470; GFI = 0.971, RMSEA = 0.000, CFI = 1).

**BMI categorical post hoc analyses**

To further explore the link between BMI-related variation in the association between the functional connectivity in the insulae networks and visceral adiposity, we performed second-level full factorial models (one for each of the insulae seeds), with visceral adiposity as the unique predictor of interest, in interaction with group (obese, overweight, lean). Sex and age were entered in the model as nuisance covariates. Linear contrasts were created to explore for effects driven by higher and lower BMI levels on the association between the insulae connectivity and visceral adiposity. The most relevant finding was found for the mIns seed and indicated that higher BMI levels (obese > overweight > lean) strengthened the association between visceral adiposity and the functional connectivity between the mIns and sensorimotor cortices (Table S2, Figure S3). At the within-group level, this was explained by a significant negative association between visceral adiposity and the functional connectivity of this mIns network in lean participants (Table S2), which was not significant (and trended in the
opposite direction) in the participants with overweight and obesity. Additional BMI effects are reported in the Supplemental Material (Table S2).

Discussion

Higher levels of visceral adiposity were associated with decreased functional connectivity between the mIns and a cluster involving the hypothalamus and the bed nucleus of the stria terminalis. Moreover, such decreased connectivity was predictive of increased food craving. Indeed, we observed that the relationship between mIns network connectivity and food craving was mediated by visceral adiposity levels. Visceral adiposity was also associated with an increased connectivity between the mIns and the bilateral middle frontal gyri as well as the right intraparietal cortex. Finally, in targeted region of interest analyses, we also observed greater connectivity between the rIns and the right amygdala-hippocampus complex associated with greater visceral adiposity levels.

The associations found between visceral adiposity levels and functional connectivity changes within mIns networks concur with previous neuroimaging studies showing anatomical and functional alterations in this region in relation to visceral adiposity (8,11,12). Furthermore, they support Craig’s models of insular cortex function (7,13), which emphasizes the role of this region in processing information about the body’s homeostatic state. The mIns has been indeed suggested to monitor hormonal signals (14,26,27) and gastric distention (28). In this context, our findings showed that the accumulation of visceral adiposity disrupts the functional connectivity between the mIns and HYP/BNST regions, which are important regulators of homeostatic eating.
Particularly, the positive correlation in the mIns and HYP/BNST turned into anticorrelation in those individuals with higher index of visceral adiposity levels. This pattern of anticorrelation may be interpreted in line with previous research suggesting that accumulation of visceral fat can compromise the brain’s sensitivity to interoceptive signals (17,18,32).

The mIns-HYP/BNST connectivity was also linked to food craving, and the mediation analysis performed with these variables nicely complemented our assumptions, i.e., the association between the interoceptive network connectivity and craving was in fact mediated by the presence of elevated visceral adiposity levels. This finding is highly consistent with hypotheses suggesting that, by altering the brain pathways involved in the perception of bodily signals, visceral adiposity promotes non-homeostatic reward-based behaviors (6,7,12,13). It is also in accord with preclinical studies showing that activation of adipose receptors in the hypothalamus leads to an increase in high-calorie food intake -with the opposite pattern observed under its antagonism- (18,33). However, the precise mechanism [e.g., altered sensitivity to adipose hormones or gastric distention, effects of proinflammatory adipokines; (34)] that links visceral adiposity with disturbed functional connectivity within the mIns networks cannot be clarified in the present study. Also, this finding may have implications for future studies aimed to investigate whether alterations in mIns networks significantly contribute to maintain at the long term high levels of visceral adiposity by promoting the desire to eat non-healthy high-caloric food.

Furthermore, our results supported the hypothesis that greater body visceral adiposity levels associate with an altered connectivity between the rIns and regions from the
salience network, which support its role in attention to external food-cues (14), in agreement with previous studies (11,12,16). Specifically, the increased functional connectivity between the rIns and the right amygdala in association with greater visceral adiposity replicates previous results in a large sample of healthy individuals that, as ours, spanned from normal to excessive adiposity values (35). However, the strength connectivity within this network did not show a significant association with the individuals’ food craving, a prediction based on findings of food-cue induced hyperactivation in these regions in individuals with elevated body visceral adiposity tissues (11,12). Further studies are needed to formally evaluate whether rIns networks are relevantly involved in the desire of unhealthy hypercaloric foods in individuals with higher levels of visceral fat.

We also found an unexpected association between visceral adiposity and the connectivity between the mIns and middle frontal and intraparietal cortices. This fronto-parietal system is involved in cognitive functioning and behavioral control (16,36). Although to our knowledge no previous study has reported such a functional alteration in these cortical regions in association with elevated visceral adiposity levels, this finding may be probably understood in the context of the large body of research on drug addiction. Such research has clearly shown that homeostatic information coded in the mIns can also modulate the value of specific goals (37). Furthermore, cognitive factors, which rely on the integrity of this frontoparietal system, might modulate the intensity of drug desire (37,38). In this sense, a plausible interpretation could be that, at higher levels of visceral adiposity, there may be a need to increase the connectivity strength within mIns-frontoparietal network to drive homeostatic behavior in the face of reduced homeostatic information -linked to the reduced connectivity within the mIns-
HYP/BNST network. Interestingly, in a recent report it has been shown that functional connectivity between homeostatic and cognitive centers of the brain could be modulated by insulin sensitivity (39). Future research may attempt to investigate how hormonal factors interact with the relationship between visceral adiposity and brain connectivity measurements.

Finally, at higher BMI levels we found a strengthened functional connectivity in the mIns-sensorimotor cortices. The regions of this network change its activation after glucose ingestion (14) and in response to gastric distention (40,41). Also, considering the role of the somatosensory cortex in taste processing (Small et al., 1999), an increased connectivity in this brain network at higher BMI levels may reflect a heightened implicit evaluation of taste processing (42), as well as assessment of the energetic and reward properties of appetizing and caloric foods (43). This is consistent both with our previous finding showing that increased connectivity in somatosensory cortices is linked to the food craving reported to appetizing food cues (44), as well as with studies that associate the insula with food craving (45–47).

Altogether, our findings show that visceral adiposity is linked to alterations in the functional connectivity of two aspects of the insula: the middle dorsal insula, which tracks homeostatic information, and the rostral insula, which codes stable representations of food properties (14). Visceral fat mediates the association between the “homeostatic network” and food craving. These results suggest that accumulation of visceral fat can disrupt the normal function of the brain’s interoceptive network and fuel (externally-driven) food cravings. These findings need to be appraised in the context of study’s limitations. First, results are correlational and thus animal models and
longitudinal studies are still needed to examine their causal role. In addition, although we used a well-validated measure of visceral fat (i.e., bioimpedance), there are some controversies, and current studies point that MRI allows greater precision in localization of fat depots (21,48). Notwithstanding the limitations, our findings may have important implications for obesity treatment. On the one hand, they suggest that diet or surgical based reductions of visceral fat can enhance interoceptive processing and control food cravings. In addition, they provide support for further testing of cognitive-behavioural interventions that focus on boosting interoceptive skills.
Acknowledgements: We acknowledge Cristian Rojas and Ismael Muela for assistance in recruitment and data collection, and to all participants in the study.

Conflict of Interest Statement: The authors declare no conflict of interest. This study has been funded by the Project Grant NEUROCOBE (PI-HUM-6635) from the Andalusian Council of Innovation, Science and Industry to Dr. Soriano-Mas is funded by a ‘Miguel Servet’ contract (CPII16/00048), and Dr. Oren Contreras-Rodriguez is funded by a ‘Sara Borrell’ postdoctoral contract (CD14/00246) from the Carlos III Health Institute (ISCIII) and by a “PERIS” postdoctoral contract (SLT006/17/00236) from the Catalan Government. Juan Francisco Navas is funded by a FPU predoctoral fellowship (FPU13/00669) from the Spanish Ministry of Education, Culture and Sport. CIBERSAM and CIBEROBN are both initiatives of ISCIII.


common gustatory and interoceptive representation in the human mid-insula.


30. Dumont EC, Mark GP, Mader S, Williams JT. Self-administration enhances


Figure Legends

Figure 1. Positive (red) and negative (blue) associations between visceral adiposity and the functional connectivity of the mIns (A) and rIns (B) seeds, controlling for BMI, age, and sex. The right hemisphere corresponds to the right side of all brain views. The color bar indicates t-values.

Figure 2. Correlations between food craving and the functional connectivity within the mIns-Hypothalamus/bed nucleus of the stria terminalis (BNST) network [A] r= -0.24, p= 0.035, and the body levels of visceral adiposity [B] r= 0.31, p= 0.006.

Figure 3. Mediation path diagram of visceral adiposity on the relationship between the connectivity in the mIns-BNST/HYP networks and food craving. Visceral adiposity shows a negative effect (path a), with decreased connectivity in the mIns-BNST/HYP network being associated with higher body visceral adiposity levels. At the same time, as body visceral adiposity levels increased the food craving increase (path b). Path c represents the direct effects of the connectivity strength in the mIns-BNST/HYP network over food craving (significant when the mediator is not into the model), whereas path c’ shows a non-significant effect (when the mediator is included). The mediation effect (path a*b) was significant. Standardized estimates and p-values (in brackets) are provided. The model is control for the influence of BMI, age, and sex.
Table 1. Demographics and clinical characteristics of the study participants.

<table>
<thead>
<tr>
<th></th>
<th>Participants (n=75)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± SD</td>
<td>range</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.09 ± 6.20</td>
<td>25 - 45</td>
<td></td>
</tr>
<tr>
<td>Education (years)</td>
<td>17.79 ± 3.74</td>
<td>9 - 25</td>
<td></td>
</tr>
<tr>
<td>Sex (men/women)</td>
<td>34(45.3%)/41(54.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>3.61 ± 1.68</td>
<td>1 - 6</td>
<td></td>
</tr>
<tr>
<td>BMI baseline (kg/m2)</td>
<td>26.34 ± 5.04</td>
<td>19 - 38.3</td>
<td></td>
</tr>
<tr>
<td>Visceral adiposity (index)</td>
<td>5.61</td>
<td>1 - 19</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>4.39</td>
<td>1 - 10</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>7.09</td>
<td>1 - 19</td>
<td></td>
</tr>
<tr>
<td>Food craving RM (VAS 0-10)</td>
<td>0.62 ± 1.19</td>
<td>-2 - 3.8</td>
<td></td>
</tr>
<tr>
<td>Appetizing Food liking catering (VAS 0-10)</td>
<td>7.61 ± 1.27</td>
<td>2.8 - 10</td>
<td></td>
</tr>
<tr>
<td>Standard Food liking catering (VAS 0-10)</td>
<td>7.14 ± 1.16</td>
<td>2.78 - 9</td>
<td></td>
</tr>
<tr>
<td>Hunger before fMRI (VAS 0-100)</td>
<td>16.82 ± 19.07</td>
<td>0 - 78</td>
<td></td>
</tr>
<tr>
<td>Hunger after fMRI (VAS 0-100)</td>
<td>42.37 ± 26.27</td>
<td>0 - 94</td>
<td></td>
</tr>
</tbody>
</table>

VAS = Visual Analog Scale

Mean and SD values are reported except for sex, where absolute numbers and percentages are provided. The socioeconomic status was determined using a questionnaire that codes it from 1-6 (1=0-600€; 2=601-1000€; 3=1001-1500€; 4=1501-2000€; 5=2001-2499€; 6=>2500€).
Table 2. Significant associations between visceral adiposity and the functional connectivity of the dorsal-middle insula (mIns), and the rostral insula (rIns) in all participants (n=75) as assessed by regression analyses.

<table>
<thead>
<tr>
<th>Seed</th>
<th>Brain region</th>
<th>x, y, z</th>
<th>t</th>
<th>CS</th>
<th>(P_{\text{unc}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>mIns</td>
<td>Middle frontal gyri</td>
<td>52, 18, 34</td>
<td>4.6</td>
<td>323</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-42, 10, 52</td>
<td>4.9</td>
<td>176</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Intraparietal cortex</td>
<td>42, -52, 40</td>
<td>5.0</td>
<td>255</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Hypothalamus/BNST</td>
<td>4, 2, -4</td>
<td>4.7</td>
<td>338</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>rIns</td>
<td>Amygdala</td>
<td>34, 2, -24</td>
<td>3.8</td>
<td></td>
<td>0.014*</td>
</tr>
</tbody>
</table>

Anatomical coordinates (x, y, z) are given in Montreal Neurological Institute (MNI) Atlas space. Abbreviations: BNST, bed nucleus stria terminals. Results for the mIns surpassed a threshold of \(P<0.001\), and 880 mm\(^3\) (101 voxels). Results for the rIns survived small-volume corrections of \(P_{\text{FWE}}<0.05\) and survive corrections for the multiple target regions used (\(P_{\text{FWE}}=0.05/3\) target brain regions= 0.016).
Visceral Adiposity (M)

Path a
-0.37 (p=0.001)

Connectivity mIns – BNST/HYP (X)

Path a*b
-0.063 (p=0.002)

Path b
0.31 (p=0.004)

Food craving (Y)

Path c: -0.24 (p=0.035)

Path c’: -0.11 (p=0.241)