Cerebral Activations During Viewing of Food Stimuli in Adult Patients with Acquired Structural Hypothalamic Damage: a Functional Neuroimaging Study

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Original Article

Abbreviated title: fMRI responses to visual food cues in acquired hypothalamic damage

Keywords: hypothalamic obesity; weight gain; hypothalamus; fMRI, insula

Word count: main text 3673, abstract 241. *Tables*: 3. *Figures*: 3 (plus one supplementary figure)

Conflict of interest: The authors have no conflict of interest to declare

ABSTRACT

BACKGROUND/OBJECTIVES: Obesity is common following hypothalamic damage due to tumours. Homeostatic and non-homeostatic brain centres control appetite and energy balance but their interaction in the presence of hypothalamic damage remains unknown. We hypothesized that abnormal appetite in obese patients with hypothalamic damage results from aberrant brain processing of food stimuli. We sought to establish differences in activation of brain food-motivation and reward neurocircuitry in patients with hypothalamic obesity (HO) compared to patients with hypothalamic damage whose weight had remained stable.

SUBJECTS/METHODS: In a cross-sectional study at a University Clinical Research Centre**,** we studied 9 patients with HO, 10 age-matched obese controls (OC), 7 patients who remained weight-stable following hypothalamic insult (HWS), and 10 non-obese controls (NOC). Functional magnetic resonance imaging was performed in the fasted state, 1 h and 3 h after a test meal, while subjects were presented with images of high-calorie foods, low-calorie foods and non-food objects. Insulin, GLP-1, PYY and ghrelin were measured throughout the experiment and appetite ratings recorded.

RESULTS: Mean neural activation in the posterior insula and lingual gyrus (brain areas linked to food motivation and reward value of food) in HWS were significantly lower than in the other 3 groups (P = 0.001). A significant negative correlation was found between insulin levels and posterior insula activation (P = 0.002).

CONCLUSIONS: Neural pathways associated with food motivation and reward-related behaviour, and the influence of insulin on their activation, may be involved in the pathophysiology of HO.

INTRODUCTION

Weight gain and obesity are common sequelae of hypothalamic damage secondary to e.g. hypothalamic tumours or craniopharyngiomas.[1,2] Hypothalamic obesity (HO) is an acute weight gain following such damage despite adequate treatment of associated hormone deficiencies, and is typically clinically significant, difficult to predict and refractory to treatment. The neurobiology of HO remains unclear.

Control of appetite depends on interacting homeostatic and non-homeostatic (cognition, emotion and reward) systems.[3] The main homeostatic brain regions regulating feeding and body weight are the hypothalamus, brainstem (especially the midbrain ventral tegmental area [VTA]) and nucleus accumbens, while the cortico-limbic and higher cortical regions are important in the processing of environmental cues, the hedonic drive to eat and the rewarding properties of food. The interactions between these two systems in humans remain poorly characterized. Functional brain imaging (fMRI) has been used to explore these by identifying brain areas that are differentially activated by alteration of the feeding state under different clinical and experimental conditions.

We hypothesized that abnormal appetite in HO results from aberrant processing of food stimuli in the neural pathways that guide reward-related behaviour, and which may assume a dominant role following hypothalamic damage. Our main objective was to establish differences in activation of food-motivation and reward neurocircuitry in HO compared to patients whose weight had remained stable following hypothalamic injury, using fMRI to measure brain responses to visual food stimuli before and after a standardized meal.

SUBJECTS AND METHODS

Participants

We studied 36 participants: 9 obese patients with hypothalamic damage (HO), 7 weight-stable non-obese patients with hypothalamic damage (HWS), and 20 healthy BMI-matched volunteers [10 non-obese controls

(NOC) and 10 obese controls (OC)] of similar age and sex. Our study protocol approved by the Northwest Research Ethics Committee, (09/H1001/4) had the following exclusion criteria for all participants: history of eating disorder, psychiatric disorder , diabetes mellitus (type 1 or 2), current (or within last 3 months) use of certain centrally acting medication (such as psychotropic or antidepressant medication, sibutramine, rimonabant that are known to influence feeding behaviour), history of traumatic brain injury, current history of excess alcohol consumption, genetic forms of hypothalamic obesity (such as Prader-Willi syndrome, Biedl-Bardet syndrome) and current history of substance abuse or addiction. Patients were recruited from specialist neuroendocrine clinics in Liverpool, UK. All had undergone treatment for hypothalamic tumours or adjacent tumours compressing or invading the hypothalamus which included 9 pituitary macroadenomas, 6 craniopharyngiomas and 1 hypothalamic glioma, with grade 2 hypothalamic damage (Saint-Rose C et al. grading) determined by a neuroradiologist. HO was defined as body mass index (BMI) $\geq$30 kg/m^2 at latest clinical follow-up which had increased $\geq$2 kg/m^2 since tumour diagnosis. HWS patients had BMI $<$30 kg/m^2 which had not increased $>$2 kg/m^2 since diagnosis. All patients were on adequate pituitary hormone replacement therapy; based on standard dynamic endocrine testing 12 patients had cortisol deficiency and were receiving hydrocortisone, 10 had secondary hypothyroidism treated with thyroxine and 6 had permanent central diabetes insipidus treated with desmopressin; all pre-menopausal women with secondary hypogonadism were on hormone replacement therapy and 6 hypogonadal male patients were treated with testosterone. Fourteen patients had severe growth hormone deficiency of whom 12 were receiving replacement therapy, the other two, being asymptomatic as assessed by the QOL-AGHDA questionnaire, did not qualify for treatment under UK guidelines. Healthy volunteers were recruited by advertisement and categorised as obese if BMI $\geq$30 kg/m^2. Written informed consent was obtained from each participant.

Study design

Participants fasted from 2200 the previous night and underwent fMRI at 0900. Blood samples were collected before the baseline scan, following which participants consumed a breakfast meal of porridge and orange juice constituting 25% of calculated basal metabolic rate. fMRI was repeated 1 h after breakfast after which participants rested quietly for 2 h undergoing blood sampling and appetite assessment. fMRI was performed again 3 h after breakfast. Blood samples were taken before and 15, 30, 60, 120 and 180 min after breakfast and at the end of all scanning, for measurement of insulin, glucagon-like peptide-1 (GLP-1), ghrelin, and Peptide YY (PYY). Visual Analogue Scale (VAS) ratings of hunger, fullness, and desire to eat were completed by the participants in the fasting state and at the end of each scanning session.

MRI acquisition

MR images were obtained using a Siemens 3 Tesla Trio (Erlangen, Germany) and eight-channel head coil. fMRI used echoplanar EPI (TR 3000 ms, TE 30 ms, flip angle 90˚, FOV 192×192 mm^2, 56 oblique 2 mm slices with slice gap 0.8 mm, voxel 3×3×3 mm^3). Whole brain anatomical T_1-weighted MRI used MDEFT (TR7.92 ms, TE 2.48 ms, flip angle 16˚, FOV 256×240, 180 1mm slices, voxel 1×1×1 mm^3).

fMRI activation task

The task presented images of high-calorie foods (e.g. sausage rolls, doughnuts), low-calorie foods (e.g. steamed salmon with vegetables, mixed fruit salad) or non-food objects (e.g. shoes, toy cars, cycle helmet). Food photographs were included based on a questionnaire where participants were asked if the food shown was high or low energy, and to rate its pleasantness (hedonic value); photographs included were those with the greatest agreement on energy content and judged the most pleasant. Images were presented using Presentation software (https://nbs.neuro-bs.com). Each block of 4 lasted 16 s and consisted of either high-calorie foods, low-calorie foods, or non-food objects, with a 6 s rest period showing a fixation cross between blocks. Each condition (high-calorie foods, low-calorie foods, objects) appeared once per cycle in random order, for a total of 8 cycles, with no duplication of images (Figure 1).

Image analysis

Pre-processing and statistical analyses used SPM8 (Statistical Parametric Mapping software package, Wellcome Department of Cognitive Neurology, London, UK: http://www.fil.ion.ucl.ac.uk/spm). Slice-timing correction was followed by realignment to correct for head movement. A mean functional image was constructed from the realigned images for each participant, and co-registered to the Montreal Neurological Institute (MNI) EPI template in SPM8. The resulting pixel size in standard stereotaxic coordinates was 2×2 mm^2, with interplane distance 2 mm. The normalized images were smoothed with an isotropic Gaussian kernel of 6×6×6 mm^3 FWHM to compensate for variations in brain size and gyral pattern.

Biochemical measurements

All samples were assayed in duplicate in one batch. Blood for measurement of GLP-1, PYY and ghrelin was collected in tubes containing 50 µl aprotinin to prevent proteolytic degradation, centrifuged at -4°C and plasma stored for analysis at -80°C. Insulin was measured using a Siemens Immulite 2000 Immunoassay. Active GLP-1 and active ghrelin were measured using commercial enzyme-linked immunoassays (ELISA) (Millipore, Billerica, USA); the standard curve range for GLP-1 was 0.8-100 pmol/l and inter- and intra-assay precisions were 8% and 7% respectively, while the corresponding range for ghrelin was 10-2000 pg/ml and inter- and intra-assay precisions were 10-16% and 7-10%. PYY (3-36) was measured using a commercial ELISA (Phoenix Pharmaceuticals Inc, Burlingame, USA); standard curve range was 0.06-100 ng/ml.

Statistical analyses

The smoothed normalised functional images were included in the first level design matrix in SPM8. For each participant, the contrast between all foods and objects was selected to remove activation related to visual perception and object recognition. The resulting single contrast images (1 per participant) were entered into a one-sample t-test (second level analysis) to determine activation to all foods across all subjects. Results were corrected for multiple comparisons using a false discovery rate (FDR) of $P \leq 0.05$ and a cluster size of $k \geq 20$. Regions of interest (ROIs) were defined using MarsBaR (http://marsbar.sourceforge.net/); only ROIs with cluster size $k \geq 3000$ voxels were used in subsequent analysis. The 6 significant clusters are shown in Table 1. Contrast values for high- and low-calorie foods in each of these 6 ROIs were defined using MarsBaR for each of the 3 sessions using each participant's first level design matrix, generating 6 contrast values per participant. Subsequent statistical analysis was performed in SPSS v.17. Six linear mixed-effects models were performed for the 6 activation clusters. For each, ROI contrast values for high- and low-calorie foods for each of the 3 sessions were entered as the outcome variable. The grouping factors *weight stable* (*NOC+HWS) vs. obese (OC+HO)*, *controls* (*NOC+OC) vs. patients (HWS+HO)* and the *interaction* between these were entered as predictor variables along with the variables *session* (fasting, 1 h and 3 h post meal) and *high/low-calorie foods*. To analyse contributions of age, sex and other variables to effects of grouping factors, two-way analyses of covariance (ANCOVA) were performed with select brain activations as dependent measures and age, sex, PYY, BMI and VAS scores as covariates.

Areas under the curve (AUC) for ghrelin, GLP-1, PYY and insulin responses were calculated by trapezoidal integration using GraphPad Prism-5 software. A linear mixed-effects model was performed using the outcome variable *ghrelin AUC* and the predictor variables *weight-stable vs. obese group*, *control vs. patient group*, and *session* (at 3 levels). Similar models were performed using outcome variables *GLP-1 AUC*, *PYY AUC* and insulin *AUC* and the VAS ratings *hunger*, *fullness* and *desire to eat*. $P<0.05$ (two-tailed) was taken as significant.

RESULTS

Nine patients with HO [mean (SD) BMI 37.7(5.4) kg/m^2, age 47(15) y], 10 age-matched obese controls (OC) [BMI 38(6) kg/m^2], 7 patients who remained weight-stable following hypothalamic insult (HWS) [BMI 26.9(2.3) kg/m^2, age 57(17) y], and 10 age-matched non-obese controls (NOC) [BMI 26(3) kg/m^2] were studied.

fMRI data

Effects of picture types (high- and low-calorie foods, objects)

Across all participants and scanning sessions there were 6 ROIs with significantly greater activation for food images compared to objects (Table 2): left-hemisphere posterior insula and middle frontal gyrus, and right-hemisphere lingual gyrus, precentral gyrus, anterior cingulate and posterior cingulate gyrus. Mean activation across the 6 regions for high- and low-calorie foods is given for each of the 4 groups in Table 2. The activation maps are shown in Figure 2.

No significant effect was found for high/low-calorie foods in any of the 6 linear mixed effects models (*P*>0.05). Potential interactions between patient/control group, lean/obese group and high/low-calorie foods were considered in each model by adding the product of the corresponding two variables as an additional explanatory variable; none significantly improved the fit (*P*>0.05) and were subsequently excluded.

Between-group comparison

In 5 of these 6 brain areas obese participants (HO+OC) showed greater activation in response to high-calorie foods than non-obese participants (HWS+NOC) (Table 2). Box plots for each of the 6 ROIs separated by patient/control and lean/obese group are shown in Figure 3.

The linear mixed-effects model showed a significant difference in activation of the lingual gyrus (*P*=0.001; coefficient -0.34, SE 0.1, 95% CI: -0.53, -0.15) and posterior insula (*P*=0.001; coefficient -0.2, SE 0.06, 95% CI: -0.33, -0.08) between *weight-stable (HWS+NOC) vs. obese (HO+OC) groups*. The activation cluster in insula, having spatial maximum in posterior insula, also spread to middle insular cortex. The interaction between the groups *weight-stable* (*HWS+NOC) vs. obese (HO+OC)* and *controls* (*NOC+OC) vs. patients (HO+HWS)* was significant for lingual gyrus (*P*<0.001; coefficient 0.47, SE 0.13, 95% CI: 0.22, 0.73) and posterior insula (*P*=0.028; coefficient 0.19, SE 0.08, 95% CI: 0.02, 0.35). Activation for both high- and low-calorie foods in lingual gyrus and posterior insula was weaker in HWS than in HO and controls (OC+NOC) (Table 3).

None of the covariates (age, sex, PYY, BMI or VAS scores) showed significant covariation in either posterior insula or lingual gyrus. Further, the interaction effects were significant even with inclusion of covariates. We conclude that the interactions between *weight-stable (HWS+NOC) vs. obese (HO+OC)* and *controls vs. patients* seen in posterior insula and lingual gyrus were not caused by individual or group differences in age or other variables.

Post-hoc pair-wise comparisons were performed for the variable session (at 3 levels: fasted, 1 h and 3 h post-meal) in each linear mixed-effects model. *Session* was significant for lingual gyrus ($F_{(2, 138)}$=4.542, *P*=0.012), posterior insula ($F_{(2,151)}$=3.024, *P*=0.05) and posterior cingulate gyrus ($F_{(2,148)}$=3.556, *P*=0.03). Pair-wise comparisons revealed a significant difference in activation across posterior insula between the fasted state and 3 h post-meal (*P*=0.04; mean difference 0.12, SE 0.06, 95%CI: 0.004,0.23) and between 1 h and 3 h post-meal (*P*=0.05; mean difference 0.09, SE 0.05, 95%CI: -0.001,0.18), with greater activation in the fasted state and 1 h compared to 3 h post-meal. Across all groups, activation of the lingual gyrus in the visual cortex was greater fasted compared to 3 h post-meal (*P*=0.003; mean difference 0.24, SE 0.8, 95%CI: 0.08, 0.40). Activation was weaker in the posterior cingulate gyrus in the fasted state compared to 1 h post-meal (*P*=0.012; mean difference -0.15, SE 0.06, 95%CI: -0.27,-0.034).

The linear mixed-effects models showed a significant effect for *control vs. patient group* where ghrelin was the outcome variable ($F_{(1,27)}$=5.245, *P*<0.03), with patients (HO+HWS) having higher ghrelin than controls (OC+NOC) (coefficient -1.06, SE 0.5, 95% CI: -2.0, -0.1); this group effect was not significantly associated with levels of PYY, GLP-1 or insulin (*P*>0.05). The effect *weight-stable vs. obese group* was statistically significant for the model where PYY was the outcome variable ($F_{(1,27)}$=8.99, *P*=0.006), with obese individuals having higher PYY than weight-stable individuals (coefficient -0.24, SE 0.1, 95% CI: -0.4,-0.1); this group effect was not significantly associated with levels of GLP-1, ghrelin or insulin (*P*>0.05).

Relationship between insula activation and hormonal parameters

ANCOVA was used to assess activation across the insula cortex while controlling for ghrelin, GLP-1, PYY and insulin. Predictor variables were selected stepwise. Only insulin was significantly associated with posterior insula activation (*P*=0.04), with negative significant correlation between insulin level and posterior insula activation (ß = -0.004, *P*=0.002) such that a 1 U/l increase in insulin corresponds to a 0.004 decrease in insula activation. Insulin AUC for the four subject groups is shown in supplementary figure.

Appetite VAS ratings

Results from the linear mixed-effects models showed higher VAS ratings for *hunger* ($P<0.05$; coefficient 8.37, SE 4.3) and *desire to eat* ($P=0.04$; coefficient 9.01, SE 4.3, 95% CI: 0.45,17.6) in *obese participants (HO+OC)* compared to *non-obese (HWS+NOC)* throughout the whole experiment irrespective of presence of hypothalamic damage.

DISCUSSION

Viewing high-calorie food-related stimuli, weight-stable patients with hypothalamic damage (HWS) showed significantly less brain activation in regions linked to processing of interoceptive inputs and modulation of food motivation behaviour (e.g., the posterior and middle insula),[4] and in regions linked to reward value for food (e.g the lingual gyrus). In patients with HO, enhanced activation of food motivation and reward neurocircuitry is accompanied by increased hunger and desire to eat, potentially influencing food-seeking behaviour and leading to higher food intake than patients who do not gain significant weight. In both these groups (HO and HWS) the hypothalamic centre of energy homeostasis is damaged. Our findings suggest that in the HWS patients there may be greater preservation of the functional and anatomical connectivity between the brain food reward processing network and the extra-hypothalamic homeostatic neurocircuitry (such as the midbrain VTA and the nucleus accumbens) allowing a more coordinated response between the homeostatic and reward networks that regulate feeding behaviour and energy balance.

Of the 6 brain regions with significantly greater activation when viewing food images compared to objects, 2 regions (insula and anterior cingulate cortex [ACC]) were first described in Tataranni's seminal PET study of hunger and satiety in humans[5] and have been identified in multiple studies since. The lingual gyrus [6] has also been identified as important in neuroimaging studies of obesity. Some of these regions determine the incentive/reward value of food,[7,8,9] some are linked to meal termination[5,10] and satiation,[5] and some with liking.[10] Further studies have shown differential activation patterns in these brain regions in obese compared to lean participants.[11,15] Although the striatal region (dorsal and ventral striatum) has been identified as an important area governing food intake and perception of food reward, we have not replicated this finding in accordance with other fMRI and PET studies. [3,4,7,9,13,15]

The greater activation in the lingual gyrus (which has been linked to the reward value of food) we observed in response to high-calorie foods in all groups compared to HWS accords with a previous PET finding[11] that obese males have greater decrease in regional cerebral blood flow in this region compared to lean following satiation with a liquid meal. Also consistent with our findings, Rothemund et al[12] found increased activation in the left lingual gyrus (and also the insula) when viewing high-calorie foods, in obese compared to lean individuals.

The posterior insular cortex is critical in appetite and feeding, and has connections with the thalamus,[13,14] hypothalamus,[13] orbitofrontal cortex,[14] prefrontal cortex (PFC) and amygdala. Posterior insula activity has been reported to increase with hunger[5,8,9] and decrease with satiation[11,15] and overfeeding[16] by decreasing the perceived salience/reward value of food stimuli.[10,17]

The insula promotes food intake and is inhibited by areas involved in meal termination, such as the PFC.[11,15] In our HWS group its connections with other important brain areas (especially the extra-hypothalamic homeostatic neurocircuitry such as the VTA and the nucleus accumbens) may have been better preserved following hypothalamic damage; this may explain the pattern of activation in insula in response to high-calorie food stimuli, which is similar to the pattern previously observed, including in lean participants .[11,15] Our finding of greater insula activation in HO compared to HWS agrees with findings in obese compared to lean cohorts without hypothalamic damage.[11,12,15,18]

The neurochemical/neuroendocrine processes underlying this differential pattern of brain activation in insula and lingual gyrus in patients who remain weight-stable compared with those who develop HO, remain speculative. The significant covariance effect between posterior insula activation decreases and increased insulin, which accords with a previous PET study,[5] is suggestive. There is animal evidence that insulin acts centrally to reduce the reward properties of food,[19] and intranasal insulin administration reduces food intake in humans,[20] suggesting that it may facilitate long-term regulation of food intake and energy balance by acting as an anorectic signal. Notably, insulin increases neuronal firing in the insula in rats,[21] and intranasal insulin administration in healthy volunteers increases neuronal activation in the insula,[22] a finding which differs from the present and previous studies,[5] and points to a potential fractionation of brain responses to insulin in the absence of an adequate peripheral insulin response. Our findings suggest that the insula responds differently to insulin signals in weight-stable (HWS) and obese hypothalamic patients (HO). Although insulin levels were similar in both groups (supplementary figure), insular activation was greater in HO than HWS, perhaps suggesting a preserved negative association between plasma insulin level and insula activation in the latter. None of the changes of PYY, GLP-1 in the 4 groups were associated with the differential brain activation patterns observed in the 6 ROIs, and more specifically in the insula and lingual gyrus which emerged as the regions of hypoactivation in the HWS group. This does not support an aetiological role for these appetite and satiety-related signals in the pathogenesis of HO.

PYY is a possible mediator of postprandial satiety. We have shown that HO patients have fasting levels of total PYY similar to obese controls,[23] and fail to exhibit an immediate and sustained post-meal rise. Intriguingly, PYY levels were greater in the obese participants compared with the non-obese, in contrast to previous reports.[24,25] Differences in experimental design, macronutrient and energy content of the test meals (which we based on individual calculated basal metabolic rate) may account for this disparity.

Leptin acts on neural circuits governing food intake to diminish perception of food reward while enhancing the response to satiety signals.[26,27] Although we found similar fasting leptin levels in HO and obese controls,[23] it would be interesting to study differences in dynamic test-meal responses of circulating leptin between HO and HWS and their correlation with the differential patterns of activation in the insula and lingual gyrus.

Our study has limitations. Previous studies have described a difference in insula activation between males and females following satiation,[28] so our mixed gender groups may have obscured some differences between pre- and post-prandial time-points. Our stringent statistical threshold may have reduced the number of areas of significant between-group difference; however, it adds additional weight to our positive findings. We also were not able to control for handedness or timing of menstruation in our female participants, due to the complex nature of the study groups involved. Ideally, we would have used a homogenous patient group, with one underlying histological diagnosis, but the limited numbers of patients seen in any single centre made it necessary to accept a more heterogeneous patient group.

In keeping with all other fMRI studies, artefacts can cause lack of homogenous image quality in some brain regions. It was not possible to personalise the food photographs to an individual's food preferences. Our study, however, used a reasonably physiological overnight fasting period, and the low- and high-calorie food photographs were of comparable hedonic value, taken on a standardized background, and with good visual variability. In summary**,** we have shown that neural pathways associated with food motivation and reward-related behaviour in response to food are differentially activated in patients with HO and in those who do not experience weight gain after hypothalamic damage. We have not been able to demonstrate in the small numbers of patients we have studied, correlation of meal initiation and termination signals such as ghrelin, PYY and GLP-1 with brain activation patterns, although we have shown that high plasma insulin levels correlated strongly with a reduction in the perceived reward properties of food. It is clear that the insula and lingual gyrus are an integral part of the network of brain areas involved in processing food stimuli. A comparatively weak posterior insula and lingual gyrus activation in the HWS patients may 'protect' these individuals from weight-gain. As this is a preliminary study reporting preliminary results, we are not suggesting a causal link between differential activation patterns of these regions and increased food intake; instead, we are shedding some light on potential disturbances of neuro-circuitry that may underlie the pathogenesis of this complex entity hypothalamic obesity, which remains poorly understood and poorly prevented and managed.

Disentangling the neurochemical/neuroendocrine processes underlying this differential pattern of brain activation in the insula and lingual gyrus may help in understanding the mechanisms underpinning weight-gain both in HO and in simple obesity in the general population. Further research into the pathophysiology of weight-gain in this interesting group of patients is encouraged, potentially in a large multi-centre study.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ACKNOWLEDGEMENTS

We thank Val Adams and Bill Bimson (MARIARC) for their help with the fMRI procedures, Nicola Williams, Neil Molyneux and Peter Taylor for their help with the purchase, preparation and taking of the food photographs and Shirley Cooper for her help during the study days. We would also like to thank all patients and healthy volunteers who participated in this study.

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FIGURE LEGENDS

Figure 1 Schematic representation of the block experimental design. Each rectangle represents a 16 sec period during which 4 pictures of the same category have been presented for 4 s each.

Figure 2 Neuronal activation across the 6 ROIs for the contrast *high- and low-calorie foods vs. objects*. Regions are shown in sagittal, coronal and axial planes, rendered on the surface of a single-subject template supplied by SPM8. Talairach coordinates are given (x, y, z) for the most significant voxel in the cluster. L = left hemisphere, R = right hemisphere. Colour corresponds to T-scores.

Figure 3 Box plots displaying neuronal activation in each of the 6 ROIs in response to high-calorie foods (as an average of all 3 sessions) in each of the 4 subject groups (from left to right): NOC, non-obese controls; OC, obese controls; HWS, weight-stable patients with hypothalamic damage; HO, obese patients with hypothalamic damage. Light grey bars represent weight-stable groups (HWS+NOC), dark grey bars obese groups (HO+OC).

Figure 4 (Supplementary) Box plots for integrated (AUC) GLP-1 (pmol/l), PYY (ng/ml), ghrelin (pg/ml) and insulin (mIU/ml) measurements in each of the 4 subject groups (from left to right): NOC, non-obese controls; OC, obese controls; HWS, weight-stable patients with hypothalamic damage; HO, obese patients with hypothalamic damage. Light grey bars represent weight-stable groups (HWS+NOC) dark grey bars obese groups (HO+OC).