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

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25 **ABSTRACT**

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

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

39 **RESULTS:** Mean neural activation in the posterior insula and lingual gyrus (brain areas linked to food
40 motivation and reward value of food) in HWS were significantly lower than in the other 3 groups ($P =$
41 0.001). A significant negative correlation was found between insulin levels and posterior insula activation (P
42 = 0.002).

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

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47

48 **INTRODUCTION**

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

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

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

67

68 **SUBJECTS AND METHODS**

69 **Participants**

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

72 (NOC) and 10 obese controls (OC)] of similar age and sex. Our study protocol approved by the Northwest
73 Research Ethics Committee, (09/H1001/4) had the following exclusion criteria for all participants: history of
74 eating disorder, psychiatric disorder, diabetes mellitus (type 1 or 2), current (or within last 3 months) use of
75 certain centrally acting medication (such as psychotropic or antidepressant medication, sibutramine,
76 rimonabant that are known to influence feeding behaviour), history of traumatic brain injury, current history
77 of excess alcohol consumption, genetic forms of hypothalamic obesity (such as Prader-Willi syndrome,
78 Biedl-Bardet syndrome) and current history of substance abuse or addiction. Patients were recruited from
79 specialist neuroendocrine clinics in Liverpool, UK. All had undergone treatment for hypothalamic tumours
80 or adjacent tumours compressing or invading the hypothalamus which included 9 pituitary macroadenomas,
81 6 craniopharyngiomas and 1 hypothalamic glioma, with grade 2 hypothalamic damage (Saint-Rose C et al.
82 grading) determined by a neuroradiologist. HO was defined as body mass index (BMI) ≥ 30 kg/m² at latest
83 clinical follow-up which had increased ≥ 2 kg/m² since tumour diagnosis. HWS patients had BMI < 30 kg/m²
84 which had not increased > 2 kg/m² since diagnosis. All patients were on adequate pituitary hormone
85 replacement therapy; based on standard dynamic endocrine testing 12 patients had cortisol deficiency and
86 were receiving hydrocortisone, 10 had secondary hypothyroidism treated with thyroxine and 6 had
87 permanent central diabetes insipidus treated with desmopressin; all pre-menopausal women with secondary
88 hypogonadism were on hormone replacement therapy and 6 hypogonadal male patients were treated with
89 testosterone. Fourteen patients had severe growth hormone deficiency of whom 12 were receiving
90 replacement therapy, the other two, being asymptomatic as assessed by the QOL-AGHDA questionnaire, did
91 not qualify for treatment under UK guidelines. Healthy volunteers were recruited by advertisement and
92 categorised as obese if BMI ≥ 30 kg/m². Written informed consent was obtained from each participant.

93

94 **Study design**

95 Participants fasted from 2200 the previous night and underwent fMRI at 0900. Blood samples were collected
96 before the baseline scan, following which participants consumed a breakfast meal of porridge and orange
97 juice constituting 25% of calculated basal metabolic rate. fMRI was repeated 1 h after breakfast after which
98 participants rested quietly for 2 h undergoing blood sampling and appetite assessment. fMRI was performed

99 again 3 h after breakfast. Blood samples were taken before and 15, 30, 60, 120 and 180 min after breakfast
100 and at the end of all scanning, for measurement of insulin, glucagon-like peptide-1 (GLP-1), ghrelin, and
101 Peptide YY (PYY). Visual Analogue Scale (VAS) ratings of hunger, fullness, and desire to eat were
102 completed by the participants in the fasting state and at the end of each scanning session.

103

104 **MRI acquisition**

105 MR images were obtained using a Siemens 3 Tesla Trio (Erlangen, Germany) and eight-channel head coil.
106 fMRI used echoplanar EPI (TR 3000 ms, TE 30 ms, flip angle 90°, FOV 192×192 mm², 56 oblique 2 mm
107 slices with slice gap 0.8 mm, voxel 3×3×3 mm³). Whole brain anatomical T₁-weighted MRI used MDEFT
108 (TR7.92 ms, TE 2.48 ms, flip angle 16°, FOV 256×240, 180 1mm slices, voxel 1×1×1 mm³).

109

110 **fMRI activation task**

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

120

121 **Image analysis**

122 Pre-processing and statistical analyses used SPM8 (Statistical Parametric Mapping software package,
123 Wellcome Department of Cognitive Neurology, London, UK: <http://www.fil.ion.ucl.ac.uk/spm>). Slice-timing

124 correction was followed by realignment to correct for head movement. A mean functional image was
125 constructed from the realigned images for each participant, and co-registered to the Montreal Neurological
126 Institute (MNI) EPI template in SPM8. The resulting pixel size in standard stereotaxic coordinates was 2×2
127 mm², with interplane distance 2 mm. The normalized images were smoothed with an isotropic Gaussian
128 kernel of 6×6×6 mm³ FWHM to compensate for variations in brain size and gyral pattern.

129 **Biochemical measurements**

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

139

140 **Statistical analyses**

141 The smoothed normalised functional images were included in the first level design matrix in SPM8. For each
142 participant, the contrast between all foods and objects was selected to remove activation related to visual
143 perception and object recognition. The resulting single contrast images (1 per participant) were entered into a
144 one-sample t-test (second level analysis) to determine activation to all foods across all subjects. Results were
145 corrected for multiple comparisons using a false discovery rate (FDR) of $P \leq 0.05$ and a cluster size of $k \geq 20$.
146 Regions of interest (ROIs) were defined using MarsBaR (<http://marsbar.sourceforge.net/>); only ROIs with
147 cluster size $k \geq 3000$ voxels were used in subsequent analysis. The 6 significant clusters are shown in Table
148 1. Contrast values for high- and low-calorie foods in each of these 6 ROIs were defined using MarsBaR for
149 each of the 3 sessions using each participant's first level design matrix, generating 6 contrast values per

150 participant. Subsequent statistical analysis was performed in SPSS v.17. Six linear mixed-effects models
151 were performed for the 6 activation clusters. For each, ROI contrast values for high- and low-calorie foods
152 for each of the 3 sessions were entered as the outcome variable. The grouping factors *weight stable*
153 (*NOC+HWS*) vs. *obese* (*OC+HO*), *controls* (*NOC+OC*) vs. *patients* (*HWS+HO*) and the *interaction*
154 between these were entered as predictor variables along with the variables *session* (fasting, 1 h and 3 h post
155 meal) and *high/low-calorie foods*. To analyse contributions of age, sex and other variables to effects of
156 grouping factors, two-way analyses of covariance (ANCOVA) were performed with select brain activations
157 as dependent measures and age, sex, PYY, BMI and VAS scores as covariates.

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

164

165 **RESULTS**

166 Nine patients with HO [mean (SD) BMI 37.7(5.4) kg/m², age 47(15) y], 10 age-matched obese controls (OC)
167 [BMI 38(6) kg/m²], 7 patients who remained weight-stable following hypothalamic insult (HWS) [BMI
168 26.9(2.3) kg/m², age 57(17) y], and 10 age-matched non-obese controls (NOC) [BMI 26(3) kg/m²] were
169 studied.

170 **fMRI data**

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

172 Across all participants and scanning sessions there were 6 ROIs with significantly greater activation for food
173 images compared to objects (Table 2): left-hemisphere posterior insula and middle frontal gyrus, and right-
174 hemisphere lingual gyrus, precentral gyrus, anterior cingulate and posterior cingulate gyrus. Mean activation

175 across the 6 regions for high- and low-calorie foods is given for each of the 4 groups in Table 2. The
176 activation maps are shown in Figure 2.

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

182 **Between-group comparison**

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

186 The linear mixed-effects model showed a significant difference in activation of the lingual gyrus ($P=0.001$;
187 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%
188 CI: -0.33 , -0.08) between *weight-stable (HWS+NOC) vs. obese (HO+OC) groups*. The activation cluster
189 in insula, having spatial maximum in posterior insula, also spread to middle insular cortex. The
190 interaction between the groups *weight-stable (HWS+NOC) vs. obese (HO+OC)* and *controls (NOC+OC) vs.*
191 *patients (HO+HWS)* was significant for lingual gyrus ($P<0.001$; coefficient 0.47 , SE 0.13 , 95% CI: 0.22 ,
192 0.73) and posterior insula ($P=0.028$; coefficient 0.19 , SE 0.08 , 95% CI: 0.02 , 0.35). Activation for both high-
193 and low-calorie foods in lingual gyrus and posterior insula was weaker in HWS than in HO and controls
194 (OC+NOC) (Table 3).

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

200 Post-hoc pair-wise comparisons were performed for the variable session (at 3 levels: fasted, 1 h and 3 h post-
201 meal) in each linear mixed-effects model. *Session* was significant for lingual gyrus ($F_{(2, 138)}=4.542, P=0.012$),
202 posterior insula ($F_{(2,151)}=3.024, P=0.05$) and posterior cingulate gyrus ($F_{(2,148)}=3.556, P=0.03$). Pair-wise
203 comparisons revealed a significant difference in activation across posterior insula between the fasted state
204 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
205 post-meal ($P=0.05$; mean difference 0.09, SE 0.05, 95%CI: -0.001,0.18), with greater activation in the fasted
206 state and 1 h compared to 3 h post-meal. Across all groups, activation of the lingual gyrus in the visual
207 cortex was greater fasted compared to 3 h post-meal ($P=0.003$; mean difference 0.24, SE 0.8, 95%CI: 0.08,
208 0.40). Activation was weaker in the posterior cingulate gyrus in the fasted state compared to 1 h post-meal
209 ($P=0.012$; mean difference -0.15, SE 0.06, 95%CI: -0.27,-0.034).

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

217

218 **Relationship between insula activation and hormonal parameters**

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

224

225 **Appetite VAS ratings**

226 Results from the linear mixed-effects models showed higher VAS ratings for *hunger* ($P<0.05$; coefficient
227 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*
228 (*HO+OC*) compared to *non-obese (HWS+NOC)* throughout the whole experiment irrespective of presence
229 of hypothalamic damage.

230

231 **DISCUSSION**

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

243 Of the 6 brain regions with significantly greater activation when viewing food images compared to objects, 2
244 regions (insula and anterior cingulate cortex [ACC]) were first described in Tataranni's seminal PET study
245 of hunger and satiety in humans⁵ and have been identified in multiple studies since. The lingual gyrus⁶ has
246 also been identified as important in neuroimaging studies of obesity. Some of these regions determine the
247 incentive/reward value of food,^{7,8,9} some are linked to meal termination^{5,10} and satiation,⁵ and some with
248 liking.¹⁰ Further studies have shown differential activation patterns in these brain regions in obese compared
249 to lean participants.^{11,15} Although the striatal region (dorsal and ventral striatum) has been identified

250 as an important area governing food intake and perception of food reward, we have not replicated
251 this finding in accordance with other fMRI and PET studies.^{3,4,7,9,13,15}

252 The greater activation in the lingual gyrus (which has been linked to the reward value of food) we observed
253 in response to high-calorie foods in all groups compared to HWS accords with a previous PET finding¹¹ that
254 obese males have greater decrease in regional cerebral blood flow in this region compared to lean following
255 satiation with a liquid meal. Also consistent with our findings, Rothmund et al¹² found increased activation
256 in the left lingual gyrus (and also the insula) when viewing high-calorie foods, in obese compared to lean
257 individuals.

258 The posterior insular cortex is critical in appetite and feeding, and has connections with the thalamus,^{13,14}
259 hypothalamus,¹³ orbitofrontal cortex,¹⁴ prefrontal cortex (PFC) and amygdala. Posterior insula activity has
260 been reported to increase with hunger^{5,8,9} and decrease with satiation^{11,15} and overfeeding¹⁶ by decreasing the
261 perceived salience/reward value of food stimuli.^{10,17}

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

269

270 The neurochemical/neuroendocrine processes underlying this differential pattern of brain activation in insula
271 and lingual gyrus in patients who remain weight-stable compared with those who develop HO, remain
272 speculative. The significant covariance effect between posterior insula activation decreases and increased
273 insulin, which accords with a previous PET study,⁵ is suggestive. There is animal evidence that insulin acts
274 centrally to reduce the reward properties of food,¹⁹ and intranasal insulin administration reduces food intake

275 in humans,²⁰ suggesting that it may facilitate long-term regulation of food intake and energy balance by
276 acting as an anorectic signal. Notably, insulin increases neuronal firing in the insula in rats,²¹ and intranasal
277 insulin administration in healthy volunteers increases neuronal activation in the insula,²² a finding which
278 differs from the present and previous studies,⁵ and points to a potential fractionation of brain responses to
279 insulin in the absence of an adequate peripheral insulin response. Our findings suggest that the insula
280 responds differently to insulin signals in weight-stable (HWS) and obese hypothalamic patients (HO).
281 Although insulin levels were similar in both groups (supplementary figure), insular activation was greater in
282 HO than HWS, perhaps suggesting a preserved negative association between plasma insulin level and insula
283 activation in the latter. None of the changes of PYY, GLP-1 in the 4 groups were associated with the
284 differential brain activation patterns observed in the 6 ROIs, and more specifically in the insula and lingual
285 gyrus which emerged as the regions of hypoactivation in the HWS group. This does not support an
286 aetiological role for these appetite and satiety-related signals in the pathogenesis of HO.

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

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

297 Our study has limitations. Previous studies have described a difference in insula activation between males
298 and females following satiation,²⁸ so our mixed gender groups may have obscured some differences between
299 pre- and post-prandial time-points. Our stringent statistical threshold may have reduced the number of areas
300 of significant between-group difference; however, it adds additional weight to our positive findings. We

301 also were not able to control for handedness or timing of menstruation in our female participants,
302 due to the complex nature of the study groups involved. Ideally, we would have used a homogenous
303 patient group, with one underlying histological diagnosis, but the limited numbers of patients seen in any
304 single centre made it necessary to accept a more heterogeneous patient group.

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

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

326

327 **CONFLICT OF INTEREST**

328 The authors declare no conflict of interest

329

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335

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413 **FIGURE LEGENDS**

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

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

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

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

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