Link between increased satiety gut hormones and reduced food reward following gastric bypass surgery for obesity

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ABBREVIATIONS:

AMV: auditory-motor-visual, ANOVA: analysis of variance, BAND: adjustable gastric banding, BED: binge eating disorder, BMI: body mass index, BOLD: blood oxygen level dependent, BP: blood pressure, FGF19: fibroblast growth factor 19, fMRI: functional magnetic resonance imaging, FDR: false discovery rate, fROIs: functional ROI, GLP-1: glucagon-like polypeptide-1, LSD: least significant difference, MRI: magnetic resonance imaging, n/a: not applicable, NAcc: nucleus accumbens, OFC: orbitofrontal cortex, Put: putamen, PYY: peptide YY, PRT: progressive ratio task, ROI: region of interest, RYGB: Roux-en-Y gastric bypass, SEM: standard error of the mean, T2DM: type 2 diabetes mellitus, VAS: visual analogue scales.

ABSTRACT

Context: Roux-en-Y gastric bypass (RYGB) surgery is an effective long-term intervention for weight loss maintenance, reducing appetite, and also food reward, via unclear mechanisms.

Objective: To investigate the role of elevated satiety gut hormones after RYGB, we examined food hedonic-reward responses following their acute post-prandial suppression**.**

Design: Randomised placebo-controlled double-blind cross-over experimental medicine studies.

Patients: Two groups, over 5 months after RYGB for obesity (n=7-11), compared with non-obese controls (n=10), or patients after gastric banding (BAND) surgery (n=9).

Intervention: Studies were performed after acute administration of the somatostatin analogue Octreotide or saline. In one study, patients after RYGB, and non-obese controls, performed a behavioral progressive ratio task (PRT) for chocolate sweets. In another study, patients after RYGB, and controls after BAND surgery, performed a functional magnetic resonance imaging (fMRI) food picture evaluation task.

Main outcome measures: Octreotide increased both appetitive food reward (breakpoint) in the PRT (n=9), and food appeal (n=9) and reward system blood oxygen level dependent (BOLD) signal (n=7) in the fMRI task, in the RYGB group, but not in control groups.

Results: Octreotide suppressed post-prandial plasma peptide YY (PYY), glucagon-like peptide-1 (GLP-1) and fibroblast growth factor-19 (FGF19) after RYGB. The reduction in plasma PYY with Octreotide positively correlated with the increase in brain reward system BOLD signal in RYGB/BAND subjects, with a similar trend for GLP-1.

Conclusions: Enhanced satiety gut hormone responses after RYGB may be a causative mechanism by which anatomical alterations of the gut in obesity surgery modify behavioral and brain reward responses to food.

INTRODUCTION

Roux-en-Y gastric bypass (RYGB) surgery is an effective long-term intervention for weight loss maintenance in obesity (1). While RYGB and other bariatric surgeries reduce appetite, patients after RYGB also have reduced food reward responses (2-4). A reduction in food preference or appetitive behaviors for energy-dense sweet/fatty foods is reported after RYGB, but not laparoscopic adjustable gastric banding (BAND), where an inflatable band is put around the proximal stomach, or the similar vertical banded gastroplasty (2,4-6). Appetitive reward for chocolate sweets but not vegetables is reduced after RYGB as measured by the effort expended to obtain food during a progressive ratio task (PRT) (7). Behaviors seen in rodent RYGB models are consistent with such clinical findings (2,8).

The exact mechanisms behind these beneficial changes in food reward remain unclear, as RYGB combines several manipulations, including: (i) reduced gastric pouch size, (ii) gastric vagus nerve manipulation, (iii) exclusion of food from stomach and proximal small bowel, (iv) disrupted bile flow with increased plasma bile acids, (v) altered gut microbiota, (vi) aversive post-prandial effects (dumping syndrome), and (vii) enhanced post-prandial plasma concentrations of satiety gut hormones including peptide YY (PYY), glucagon-like peptide-1 (GLP-1) and fibroblast growth factor 19 (FGF19), related to earlier delivery of food to the midgut and disrupted bile flow (3,4,10-13). Post-prandial plasma PYY and GLP-1 concentrations are substantially higher after RYGB than BAND surgery (4,14,15).

We hypothesised that satiety gut hormones attenuate appetitive and anticipatory food reward responses after RYGB in humans, and therefore that acute suppression of these hormones by subcutaneous administration of the somatostatin analogue Octreotide, would

increase food reward in patients after RYGB.

In functional magnetic resonance imaging (fMRI) studies, changes in blood oxygen level dependent (BOLD) signal reveal that activation in key brain reward areas including amygdala, caudate, nucleus accumbens, anterior insula and orbitofrontal cortex (OFC) is lower after RYGB, when evaluating food pictures, compared to BMI-matched patients after BAND, despite both surgical groups having similarly low hunger ratings (4). This lower anticipatory fMRI reward response to food after RYGB was accompanied by lower high-energy food picture appeal ratings (4). Longitudinal reductions in high-energy food hedonics and reward responses using fMRI have also been reported after compared to before RYGB surgery (9).

Although PYY and GLP-1 additively increase satiety to reduce caloric intake (16,17), direct evidence for their importance in the reduced food intake after RYGB remains unproven. Antagonism of GLP-1 increases food intake and body weight after RYGB in rodents but no more than in sham-operated animals (18), while genetic disruption of GLP-1 release or GLP-1 receptor (GLP-1R) in rodent models of bariatric surgery does not attenuate weight loss (18-20). Antagonism of PYY has not consistently increased food intake and body weight in RYGB rodent models (14,18), but knockout of *Pyy* increased food intake and attenuated weight loss after RYGB in mice (21). Nevertheless a broader intervention through acute suppression of *several* plasma gut hormones including PYY and GLP-1 secretion by administration of the somatostatin analogue Octreotide does increase food intake in both rats (22) and humans (23) after RYGB. Chronic subcutaneous administration of Octreotide for dumping syndrome after RYGB also leads to weight gain (24).

Additional roles for these satiety gut hormones have recently been postulated for the changes in food reward after RYGB (4). PYY modulates resting activity in brain reward systems (25), and PYY and GLP-1 are additive in reducing BOLD signal in response to food pictures in the amygdala, caudate, putamen, nucleus accumbens, anterior insula and OFC in non-obese subjects (17). Furthermore the reduction in brain responses to food pictures with a GLP-1R agonist in non-obese and obese subjects is blocked by co-administration of a GLP-1R antagonist (26). In animal studies GLP-1R agonists reduce food intake and reward-based eating behavior, e.g. reducing the effort expended to obtain sucrose in rats using a PRT, through the nucleus accumbens and mesolimbic pathways (27).

Despite these observations, no mechanistic study has yet directly established which specific anatomical or physiological perturbation after RYGB in humans reduces food reward. The objective of this study was to: (i) investigate the effect of satiety gut hormone suppression, using acute administration of Octreotide, on food hedonic-reward responses in subjects after RYGB, using established behavioral and functional neuroimaging methodologies, and (ii) compare these responses to those of subjects without enhanced post prandial gut hormone secretion, namely obese unoperated controls or patients after BAND surgery.

MATERIALS AND METHODS

Progressive Ratio Task

Participants

Eleven patients who had previously undergone RYGB surgery were recruited from the obesity clinic at St. Columcille's Hospital, Dublin, with ten unoperated non-obese weight stable volunteers as age- and gender-matched controls. Inclusion criterion for the RYGB group was surgery more than 5 months previously. Exclusion criteria for both RYGB and control non-obese groups were: (i) smoking, (ii) pregnancy or breast feeding, (iii) significant neurological, psychiatric or cardiovascular disease including addiction, stroke and epilepsy, other than previous depression, (iv) type 1 or 2 diabetes mellitus, (v) lack of understanding of the test instructions, (vi) dislike for the test stimuli, (vii) active post-operative complications, (viii) current intolerance to sweet foods due to dumping syndrome.

Progressive ratio task visit protocol

Participants completed two 1.5 hour sessions at the Clinical Research Centre, St. Vincent University or St. Columcille's Hospitals in a within-subject double-blind randomized cross-over design (Supplementary Figure 1A), using sequential list randomization. After an overnight 12 hour fast, participants were injected subcutaneously with either 1mL Octreotide (Sandostatin, Novartis Pharmaceuticals, 100 mcg) or 0.9% saline (23) (Figure 1A). Water was allowed during the overnight fast.

Five minutes after the injection participants were asked to consume an *ad libitum* breakfast of their choice until they felt "comfortably full" to stimulate gut hormone release, and achieve similar degrees of satiety between groups. The quantity and type of food eaten was

recorded and participants were given the exact same at the second visit, which they all consumed.

One hour after breakfast, participants rated their hunger, fullness, desire to eat and nausea on a 10cm visual analogue scale (VAS) (4). Sixty-five mins after breakfast, participants sat in front of a computer screen with a plate of 20 chocolate sweets (M&M® crispy candies, Mars UK Limited, Slough UK, 4kCal each, energy: 43.7% sugars, 44.1% fat) and performed the PRT (7). An increasing number of computer mouse clicks is needed to earn a sweet. The starting ratio to get a reward was 10 clicks with a geometric increment of 2 (i.e. 10, 20, 40, etc.) until the participant stopped responding, which was the breakpoint. No food or fluid was offered after the task. Participant instructions are given in the Supplementary Methods.

Blood sampling

Following completion of the PRT, 80 mins after breakfast, venous blood samples were collected for measurement of serum insulin, plasma glucose, total GLP-1, total PYY and FGF19 (see Supplementary Methods).

Functional MRI Study

Participants

Seven weight stable patients who had previously undergone RYGB surgery, and nine weight stable patients who had undergone BAND surgery, at the Imperial Weight Centre, Charing Cross Hospital, London completed fMRI scanning, as extra visits to a previous study (see Supplementary Methods for further details) (4). Two additional new patients after RYGB completed identical study visits performing the food picture evaluation task outside the

scanner due to contraindications to MR scanning. Appeal ratings were therefore available for nine patients after RYGB, and fMRI scans for seven patients after RYGB and nine patients after BAND surgery. Exclusion/inclusion criteria were as described (4).

Scanning visit protocol

After an overnight fast (water was allowed), patients were randomized, in a double-blind cross-over within-subject design (Figure 1B), to receive, 10 minutes before breakfast, either two subcutaneous injections of 0.9% saline, or Octreotide (Sandostatin, Novartis Pharmaceuticals, 100 mcg) (23) plus a short-acting insulin to attenuate post-prandial hyperglycemia from suppression of insulin (Actrapid, Novo Nordisk, 0.075-0.10 units/kg), as two separate injections. Subjects then received a standardized milkshake breakfast (1 sachet Complan® plus 200ml whole milk giving 385kCal, energy: carbohydrate 45.6%, fat 16.0%, protein 38.4%), at t=0 min. Participants were monitored for a total of 165 minutes to ensure they did become hypoglycemic.

Food picture evaluation fMRI paradigm

During the fMRI paradigm, starting at +95 mins, four types of color photographs were presented in a block design split across two runs: (i) 60 high-energy foods (e.g. pizza, cakes and chocolate), (ii) 60 low-energy foods (e.g. salads, vegetables, fish), (iii) 60 non-food related household objects (e.g. furniture, clothing), with each category in ten blocks, and (iv) 180 Gaussian blurred images of the other pictures, as described (4,28).

Whilst each image was on display to subjects in the scanner, they were asked to immediately and simultaneously rate how 'appealing' each picture was to them using a 5 button hand-

held keypad (1=not at all, 5=a lot) (4,28).

Auditory-motor-visual control fMRI paradigm

In the 6 minute auditory-motor-visual (AMV) control task, over nine blocks, subjects performed two of the following tasks simultaneously: (i) listening to a story, (ii) tapping their right index finger once every second, or (iii) watching a 4Hz color (yellow/blue) flashing checkerboard, with each task performed in 6 blocks (4,28). This control fMRI task was used to ensure that changes in BOLD signal were not non-specific e.g. due to alterations in neurovascular coupling.

FMRI analysis

Preprocessing used the fMRI Expert Analysis Tool v5.98 software, part of the FMRIB Software Library (FSL), version 4.1 (www.fmrib.ox.ac.uk/fsl), as described (4,28).

The average (median) BOLD signal for the contrasts high-energy food > object and low-energy food > object were calculated for each subject at each visit in these functional regions of interest (fROIs): amygdala, nucleus accumbens, anterior insula and caudate nucleus (4,28). The fROIs were determined from a separate cohort of 24 overweight/obese subjects who underwent an identical protocol after fasting overnight, without administration of Octreotide/Insulin, for the food (high-energy or low-energy) > object contrast at voxel-wise false discovery rate corrected $P<0.05$, as previously described (4) (and see Supplementary Methods). Excess signal dropout precluded use of an OFC fROI. Similar functional localizers were made for the control AMV task for bilateral superior posterior

temporal gyrus (auditory), left pre-central gyrus (motor), and bilateral lingual gyrus (visual), from the same separate cohort of 24 overweight/obese subjects, as described (4).

Appetite ratings

VAS ratings (0-10 cm) of appetite and other symptoms were recorded at serial time points to measure hunger, pleasantness to eat, volume of food wanting to eat, fullness and sickness (4,28).

Blood sampling

The concentration of serum insulin, plasma glucose, insulin and total GLP-1, total PYY, and FGF19 (see Supplementary Methods for assay methods), over the scanning period was calculated using the mean of concentrations at time point +70 min (just before the fMRI scan) and time point +150 min (just after the fMRI scan).

Statistical Analysis

SPSS v21 (IBM) and Prism v5 (GraphPad Software Inc.) were used to perform the statistical analyses. Results are presented as mean ± SEM or median [interquartile range] for data that was not normally distributed (Kolmogorov-Smirnov test), and range. Significance was taken as $P<0.05$. Effect sizes are described as mean difference ± SEM, 95% confidence intervals (CI), and Cohen's d values.

Between Group comparisons used unpaired Student's t-test, Mann-Whitney U test (if data not normally distributed) or Fisher's exact test for categorical variables to examine Group differences. Within Group comparisons used paired t-test or Wilcoxon Rank Sum Test (if data

not normally distributed) to examine the response to Treatment (Octreotide vs. Saline) separately in each Group. In the fMRI study, 2-way repeated measures ANOVA including Treatment (Octreotide vs. saline) and food picture energy density (high-energy vs. low-energy food) as within subject factors was used to determine the effects of Treatment, energy density and Treatment x energy density interaction, separately within each group, with post-hoc Fisher's least significance difference (LSD) Test. Multivariate analysis was performed using all fROIs, as well as univariate analysis for each individual fROI or average signal across all fROIs.

To directly compare the effects of Octreotide between Groups, univariate 2-way repeated measures ANOVA were performed including Treatment (Octreotide vs. saline) as within subject factor, and Group as between subject factor, to determine effects of Treatment, Group and Treatment x Group interaction, with post-hoc Fisher's LSD Test. In fMRI analyses, energy density (high-energy vs. low-energy food) was also included as an additional within subject factor in a univariate 3-way repeated measures ANOVA. Given our *a priori* hypothesis of enhanced effects of Octreotide in RYGB groups, we illustrate post-hoc effects of Treatment within each group, irrespective of the Treatment x Group interaction effect.

Pearson correlation coefficients r were determined from linear regression analysis to examine the relationship between the change in outcome variables between Octreotide and saline visits, and either the variable at the saline visit, or the change in plasma gut hormones between the Octreotide and saline visits.

RESULTS

Progressive ratio task

The healthy non-obese control group and adults after RYGB were of similar age, gender ratio and ethnicity, but the RYGB group remained significantly heavier than controls (Table 1). Five of the 10 controls and five of the 11 RYGB subjects had the saline injection at the first visit.

Glucose and hormone responses

As expected, compared to saline administration, Octreotide suppressed post-prandial plasma PYY and GLP-1, serum insulin and, but also plasma FGF19, concentrations in patients after RYGB (Figure 2B-E, Supplementary Table 2). In controls this suppressive effect of Octreotide was only significant for GLP-1. However there was a significantly greater suppression of insulin and PYY with Octreotide in patients after RYGB than controls, but not for GLP-1 or FGF19 (Figure 2B,D, Supplementary Table 2).

Octreotide significantly increased post-prandial plasma glucose in patients after RYGB but not controls, though concentrations remained ≤7.7 mmol/L (≤139 mg/dL), i.e. not in the hyperglycaemic range, and there was no significant difference between groups in the effect of Octreotide on plasma glucose (Figure 2A, Supplementary Table 2).

At the Saline visit, post-prandial serum insulin and plasma PYY concentrations were greater in patients after RYGB than non-obese control subjects [insulin: mean difference RYGB minus control (95% CI) -19.0 (11.4, 26.7) mU/L, $F(1,19)=27.41$, $P<0.001$, Cohen's $d=2.29$; PYY: 128.0 (56.6, 199.3) pmol/L $F(1,19)=14.08$, $P=0.001$, Cohen's $d=1.64$], with a similar trend for plasma FGF19 [28.0 (-3.2, 59.2) pg/mL $F(1,19)=3.54$, $P=0.075$, Cohen's $d=0.82$] (Figure 2,

Supplementary Table 2). However plasma glucose and GLP-1 concentrations were not significantly different between groups [glucose: -0.4 (-1.0, 0.2) mmol/L, F(1,19)=2.39, P=0.14, Cohen's d=0.68; GLP-19.8 (-3.8, 23.3) pmol/L, F(1,18)=2.28, P=0.15, Cohen's d=0.67] (Figure 2, Supplementary Table 2).

Behavioral responses

The breakpoint of the PRT, which represents the amount of work the subject did to obtain the reward, was not significantly different between RYGB and control groups after saline administration (Figure 3, Supplementary Figure 1). There was a significantly greater effect of Octreotide in the RYGB compared to the control group, with Octreotide significantly increasing the breakpoint in patients after RYGB (Figure 3, Supplementary Figure 1), but not the control group. By contrast, Octreotide had no significant effects on VAS ratings of hunger, fullness or nausea in either group (Supplementary Table 1).

Hormone-behavior correlations

There was no significant correlation between the increase in PRT breakpoint, and the decrease in plasma PYY, GLP-1 or FGF19, with Octreotide administration (both Δ Octreotide - Saline) in patients after RYGB alone, or in the RYGB and control group combined (Supplementary Table 3).

Functional MRI study

Appeal ratings were available for nine RYGB subjects, and fMRI scans for seven RYGB subjects. The RYGB and BAND groups were of similar age, gender ratio, ethnicity, pre-operative BMI, current BMI and % body fat, % weight loss since surgery and time since

surgery (Table 1).

Glucose and hormone responses

At the Saline visit, post-prandial serum insulin, and, as expected, plasma GLP-1 and PYY concentrations were significantly greater in patients after RYGB than after BAND [insulin: mean difference RYGB minus BAND (95% CI) 5.7 (1.2, 10.3) mU/L, $F(1,16)=7.07$, $P=0.017$, Cohen's $d=1.25$; GLP-1: 12.3 (2.6, 22.0) pmol/L, $F(1,16)=7.26$, $P=0.016$, Cohen's $d=1.27$; PYY: 123.4 (12.6, 234.1) pg/mL, $F(1,16)=5.58$, $P=0.031$, Cohen's $d=1.11$] (Figure 4, Supplementary Table 2). However there was no significant difference in plasma glucose or FGF19 concentrations between RYGB and BAND groups [glucose: -0.2 (-1.2, 0.8) mmol/L, $F(1,16)=0.27$, $P=0.61$, Cohen's $d=0.25$; FGF19: 31.0 (-20.9, 83.0) pg/mL, $F(1,16)=1.60$, $P=0.22$, Cohen's $d=0.60$] (Figure 4, Supplementary Table 2).

As expected, the gut hormone suppressive effects of Octreotide in the RYGB group were similar in the fMRI study to that in the PRT study. Compared to saline administration, Octreotide significantly suppressed post-prandial plasma GLP-1, PYY and again FGF19, over the scanning period (Figure 4C-E). Interestingly, Octreotide also suppressed post-prandial GLP-1 and PYY, but not post-prandial FGF19 in the BAND group (Figure 4C-E). Octreotide had a greater suppressive effect on plasma GLP-1 and PYY in the RYGB group than BAND group, with a similar trend seen for FGF19 (Supplementary Table 4). This appears to be a consequence of higher post-prandial plasma gut hormone concentrations, particularly for GLP-1, in patients after RYGB than BAND at the Saline visit.

The co-administration of exogenous insulin avoided the potential confound of significantly

lower post-prandial serum insulin concentrations after Octreotide in both the RYGB and BAND groups (Figure 4B). In the BAND group co-administration of Octreotide and exogenous insulin did not increase post-prandial glucose concentrations (Figure 4A). However, post-prandial glucose concentrations still increased after Octreotide in the RYGB group, though concentrations were ≤9.5 mmol/L (<171 mg/dL) (Figure 4A, Supplementary Table 4). None of our patients reported any hypoglycaemic adverse events after discharge from the clinical research facility.

Food hedonic responses

Compared to saline, Octreotide significantly increased food appeal ratings in patients after RYGB (Figure 5A), but not in patients after BAND (Figure 5B). However on direct comparison, there was no significant difference in the effect of Octreotide on food appeal ratings between surgical groups (Supplementary Figure 2A, Supplementary Table 5).

BOLD signal responses

In the *RYGB* group, *multivariate* analysis including all the *a priori* fROIs (nucleus accumbens, caudate, anterior insula, amygdala) revealed that Octreotide significantly increased BOLD signal during evaluation of food pictures [$F(4,3)=13.86$, $P=0.028$], independent of the food picture energy density (energy density x Treatment interaction [$F(4,3)=0.57$, $P=0.71$]). In *univariate* analysis of individual fROIs in the *RYGB* group, Octreotide significantly increased BOLD signal during food picture evaluation averaged across all the *a priori* fROIs, and in the nucleus accumbens alone (Figure 5C,E). Interestingly a lower food appeal and BOLD signal during food evaluation after saline, was associated with a greater increase in these variables with Octreotide administration (Supplementary Figure 3).

By contrast, in *multivariate* analysis of all fROIs in the *BAND* group, Octreotide had no significant effect on BOLD signal during evaluation of food pictures in the a *priori* fROIs: [F(4,5)=0.45, P=0.77], independent of the food picture energy density (energy density x Treatment interaction [F(4,5)=0.26, P=0.89]). Similarly in *univariate* analysis of individual fROIs in the *BAND* group, Octreotide had no significant effect on BOLD signal during food picture evaluation averaged across all the *a priori* ROIs, or in the nucleus accumbens (Figure 5D,F).

However on direct comparison, there was no significant difference in the effect of Octreotide on BOLD signal averaged across all four *a priori* fROIs, the nucleus accumbens or other individual ROIs (Supplementary Figure 2, Supplementary Table 5).

Hormone-BOLD signal correlations

In the combined surgical group analysis (n=16), there was a significant positive correlation between the increase in BOLD signal to food pictures averaged across the *a priori* fROIs with Octreotide administration, and the decrease in plasma PYY with Octreotide administration (r(15)=+0.56, P=0.02, Figure 6A), with a similar trend for GLP-1 (r(15)=+0.45, P=0.08, Figure 6B), but not FGF19 (r(15)=+0.06, P=0.83).

Confounding variables

As with the PRT study, Octreotide had no significant effects in either the RYGB or BAND groups on: (i) VAS ratings of hunger or fullness (Supplementary Tables 6 and 7), (ii) BOLD signal during the control auditory-motor-visual fMRI task (suggesting a lack of non-specific

effects), (iii) motion during scanning, (iv) emotional ratings (Supplementary Tables 8 and 9), or (v) post-prandial signs and symptoms of dumping syndrome (Supplementary Tables 10 and 11).

DISCUSSION

In two cohorts of patients after RYGB using complimentary behavioral tests and functional neuroimaging, we demonstrate that lowering post-prandial plasma satiety gut hormones by acute Octreotide administration, increased the breakpoint for a chocolate sweet in a PRT, and increased food appeal ratings and brain reward system activation during evaluation of food pictures.

These measures of food reward are of direct relevance to actual habitual eating behavior, food intake and changes in body weight, though these are not examined in the current study. The magnitude of change in the breakpoint for chocolate sweets in the PRT have been correlated with the degree of weight loss after RYGB (7), and the breakpoint for different foods correlate with habitual dietary intake and energy intake in test meals (29). BOLD signal in brain reward regions, including nucleus accumbens, caudate, amygdala and insula, to anticipatory food cues correlate with pleasantness ratings of ingested food after bariatric surgery (4), and predict food intake at test meals (30,31) and weight loss during lifestyle modification (32). The caudate and nucleus accumbens, are involved in habitual and goal- and reward-directed behavior. The anterior insula incorporates the primary taste cortex, and together with the amygdala, that predominantly responds to emotions but also receives gustatory and other multi-modal sensory inputs, form part of a brain network that regulates stimulus-response association and motivation to rewards such as food.

By suppressing post-prandial release of gut hormones such as GLP-1 and PYY, Octreotide administration has an advantage over specific antagonists in attenuating the *additive* effects of several satiety gut hormones to reduce reward-related eating behavior (17). A novel

finding in both our studies was that Octreotide reduced elevated post-prandial plasma FGF19 in patients after RYGB. This could be via a direct effect on somatostatin receptors on enterocytes, or perhaps mediated indirectly through changes in other gut hormone pathways. FGF19 has a central anorexigenic action in rodents (33), in addition to roles in regulation of liver, lipid and glucose metabolism (34). Indeed weight loss after vertical sleeve gastrectomy in rodents is dependent on intact signalling at the farsenoid-X receptor (FXR) that mediates the stimulation of FGF19 secretion by enterocytes in response to bile acids (13). In our fMRI study, while increased reward system BOLD signal with Octreotide correlated with the suppression of PYY in the combined bariatric surgical groups, with a similar trend for GLP-1, this was not seen for FGF19, suggesting a more prominent role for PYY/GLP-1 in mediating these changes. Nevertheless, a potential role for FGF19 in reward-related eating behavior would be worthy of further study.

While Octreotide-induced hypoinsulinemia could contribute to the increased breakpoint in the PRT study and enhanced food reward (35-37), increases in hedonic-brain responses to food in the fMRI study were still seen despite its prevention by co-administration of exogenous insulin. However this is a potential limitation in interpretation of the PRT study. Furthermore the relative hyperglycemia seen after Octreotide in both studies would, if anything, be expected to attenuate rather than enhance such reward responses (38,39).

Activation of brain somatostatin 2 receptors stimulates feeding in rodents, raising the possibility of a direct brain effect of Octreotide (40). However the increase in PRT breakpoint for the sweet chocolate reward with Octreotide in the RYGB group was not seen in the control non-obese group, suggesting a lack of non-specific or direct central effects of the

459 somatostatin analogue. Similarly the increase in food appeal and reward system BOLD signal
460 with Octreotide in the RYGB group was not seen in the matched BAND group. However no
461 significant difference was seen between the response to Octreotide in direct comparisons
462 between the RYGB and BAND groups. This is likely related to the small number of subjects
463 studied and stringent statistics used, but also due to the fact that Octreotide does *still* have
464 an effect to reduce post-prandial PYY and GLP-1 secretion in patients after BAND. The
465 attenuation of these satiety gut hormones with Octreotide was lower in the BAND than
466 RYGB groups, related to higher post-prandial concentrations after RYGB. Together with the
467 correlation results in the combined surgical groups, these findings suggest that gut
468 hormones may have a concentration dependent attenuating effect on reward system BOLD
469 signal after bariatric surgery.
470
471 A limitation of our study is the small number and power especially in the exploratory fMRI
472 study, meaning that the findings are in need of confirmation in larger studies. This may also
473 have resulted in false positive, as well as false negative errors, for example in analysis of the
474 interaction with food energy density and lack of significance in the BAND group in the fMRI
475 study. However n=9 in the BAND group had power of 0.74-0.88 to detect similar differences
476 in BOLD signal with Octreotide as seen in the RYGB group (see Supplementary Methods).
477 Furthermore we found a similar direction of effect of Octreotide to increase food reward
478 using three different measures (PRT breakpoint; food appeal and BOLD signal in brain
479 reward system during food evaluation) in two separate cohorts of patients after RYGB. We
480 did not include an unoperated obese control group in either study that would also have been
481 of interest, particularly for the PRT study where obesity may influence the breakpoint (7).
482 However the use of a BAND control group in the fMRI study, as an extension of our previous

study (4), matched for BMI and weight loss, provided an ideal control for weight loss from a surgical intervention, but without an exaggerated satiety gut hormone response. Additionally our study investigated only the acute but not the chronic effects of satiety gut hormone suppression on food reward and therefore firm conclusions on their role in food intake and choice and long-term weight loss cannot be made.

In conclusion, acute suppression of the enhanced post-prandial responses of satiety plasma gut hormones, such as PYY, GLP-1 and FGF19, increased both appetitive food reward in a PRT, and brain-hedonic responses to anticipatory food reward using fMRI, after RYGB. These results are consistent with the hypothesis that enhanced satiety gut hormone responses after RYGB, resulting from anatomical alteration of the upper gastrointestinal tract, contribute to the reduction in brain-hedonic responses to food after RYGB. This gives new insights into the increasingly recognised impact of gut hormones on reward-based eating behavior.

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644 **TABLES**

645 **Table 1. Participant characteristics in progressive ratio task and fMRI food evaluation studies**

	Progressive ratio task study			fMRI food evaluation study [a]			
	Controls	**RYGB**	**P** [b]	**RYGB (all)**	**RYGB (scanned)** [c]	**BAND**	**P** [d]
N	10	11		9	7	9	
Age (years)	45.3 ± 3.4 (31.0, 62.0)	50.5 ± 9.2 (34.0, 68.0)	0.25	47.0 ± 1.8 (42.0, 59.0)	46.0 ± 1.0 (42.0, 50.0)	41.8 ± 3.8 (26.0, 59.0)	0.22
Gender (Male / Female)	6/4	6/5	1.00	3/6	2/5	1/8	0.58
European Caucasians, n (%)	10 (100%)	11 (100%)	1.00	9 (100%)	7 (100%)	6 (75%)	0.21
Pre-operative BMI (kg/m^2)	n/a	54.0 ± 2.8 (41.2, 71.0)	n/a	52.8 ± 2.0 (38.3, 74.6)	55.2 ± 5.3 (38.3, 74.6)	51.7 ± 4.8 (36.5, 86.2)	0.84
Current BMI (kg/m^2)	24.5 ± 0.6 (20.6, 26.9)	38.8 ± 2.0 (25.8, 49.1)	**0.02**	37.3 ± 2.6 (29.4, 48.8)	38.6 ± 3.1 (29.4, 48.8)	33.2 ± 6.3 (25.2, 43.8)	0.25
Current weight (kg)	71.8 ± 3.2 (56.1, 88.20)	110.6 ± 8.9 (63.5, 169.9)	**<0.001**	105.6 ± 6.2 (75.0, 129.3)	109.0 ± 6.4 (82.2, 129.3)	91.3 ± 4.8 (75.5, 115.1)	0.10
Current body fat (%)	ND	ND	n/a	42.0 ± 3.9 (23.1, 55.1)	41.5 ± 5.0 (23.1, 55.1)	40.2 ± 3.2 (23.6, 52.0)	0.73
Weight loss (% pre-operative weight)	n/a	27.2 ± 3.9 (3.0, 47.0)	n/a	28.3 ± 2.0 (21.1, 38.3)	29.1 ± 2.4 (21.1, 38.3)	27.4 ± 4.0 (10.0, 52.0)	0.84
Time since surgery (months)	n/a	28.4 ± 5.5 (5.6, 68.0)	n/a	15.6 ± 2.5 (5.2, 23.9)	14.2 ± 3.0 (5.2, 23.9)	15.3 ± 3.6 (4.0, 36.0)	0.95
Pre-operative DM, n (%)	n/a	2 (18%)	n/a	5 (56%)	4 (57%)	0 (0%)	**0.03**
Current DM, n (%)	0 (0%)	1 (9%)	1.00	1 (11%)	1 (14%)	0 (0%)	1.00
Pre-operative BED, n (%)	n/a	ND	n/a	2 (22%)	1 (14%)	2 (22%)	1.00
Current BED, n (%)	ND	ND	n/a	1 (11%)	1 (14%)	0 (0%)	1.00

Data presented as mean ± SEM and (minimum, maximum), or n (%).

[a] data calculated from average of the 2 visits in the fMRI evaluation study .

P value for comparison of [b] Controls vs. RYGB, or [d] RYGB (all) vs. BAND, using independent 2-tailed t-test for continuous variables or Fisher exact test for prevalence data.

[c] excluding 2 participants with contraindications to MRI.

Abbreviations: BED: binge eating disorder, BMI: body mass index, DM: type 2 diabetes mellitus, n/a: not applicable, ND: not determined.

FIGURE LEGENDS

Figure 1. Study protocols.

(A) Progressive ratio task. (B) Functional MRI food evaluation study. Note that the PRT lasted 10 minutes and all participants finished the task within this time period. The time interval between the end of the PRT and the +80 min blood sample ranged from 9 to 13 minutes.

Abbreviations: AMV: auditory-motor-visual, BP: blood pressure, fMRI: functional MRI, MRI: magnetic resonance imaging, PRT: progressive ratio task, VAS: visual analogue scales

Figure 2. Octreotide increased food reward responses in progressive ratio task.

Octreotide significantly increased the total number of clicks (breakpoint) in patients after RYGB [mean difference (95% CI) 503 (218, 788), $F(1,19)=13.66$, $P=0.002$, Cohen's $d=0.62$], but not in controls [effect size -1 (-300, 298), $F(1,19)=0.00$, $P=0.99$, Cohen's $d=0.00$]. Overall ANOVA: Group (between subject) $F(1,19)=1.53$, $P=0.23$; Treatment (within subject) $F(1,19)=6.47$ $P=0.02$; Group x Treatment interaction $F(1,19)=6.54$, $P=0.02$.

Figure 3. Octreotide suppressed post-prandial plasma gut hormones in progressive ratio task.

(A) Plasma glucose. Octreotide significantly increased plasma glucose in patients after RYGB [mean difference (95% CI) 0.6 (0.2, 1.1) mmol/L, F(1,19)=7.84, P=0.011, Cohen's d=0.84], but not in controls [0.3 (-0.2, 0.8) mmol/L, F(1,19)=1.25, P=0.28, Cohen's d=0.00]. To convert mmol/L to mg/dL multiply by 18.01.

(B) Serum insulin. Octreotide significantly decreased serum insulin in patients after RYGB [mean difference (95% CI) -22.3 (-27.7, -16.9) mU/L, F(1,19)=74.88, P<0.001, Cohen's d=2.05], but not in controls [-4.0 (-9.6, 1.7) mU/L, F(1,19)=2.15, P=0.10, Cohen's d=1.08]. To convert mU/L to pmol/L multiply by 7.175.

(C) Plasma glucagon-like peptide-1 (GLP-1). Octreotide significantly decreased plasma total GLP-1 both in patients after RYGB [mean difference (95% CI) -18.7 (-27.7, -9.6) pmol/L, F(1,18)=18.81, P<0.001, Cohen's d=0.98], and in controls [-11.0 (-20.0, -1.93) pmol/L, F(1,8)=6.50, P=0.020, Cohen's d=1.49].

(D) Plasma PYY. Octreotide significantly decreased plasma total PYY in patients after RYGB [mean difference (95% CI) -143.8 (-193.3, -94.4) pg/mL, F(1,19)=37.08, P<0.001, d=1.48], but not in controls [-30.0 (-81.8, 21.9) pg/mL, F(1,19)=1.46, P=0.24, d=0.62]. To convert pg/mL to pmol/L multiply by 0.25.

(E) Plasma fibroblast growth factor-19 (FGF19). Octreotide significantly decreased plasma FGF19 in patients after RYGB [mean difference (95% CI) -36.6 (-59.4, -13.7) pg/mL, F(1,19)=11.23, P=0.003, Cohen's d=0.91], but not in controls [-12.1 (-36.1, 11.9) pg/mL, F(1,19)=1.12, P=0.30, Cohen's d=0.47].

Data represents mean ± SEM, n=11 per group (except RYGB group GLP-1 n=10). Saline (white bars), Octreotide (black bars). *P<0.05, ***P<0.001 with post hoc Fisher's LSD test. For statistics of repeated measures ANOVA for effects of Group, Treatment and Group x Treatment interaction see Supplementary Table 2.

Figure 4. Octreotide suppressed post-prandial plasma gut hormones in fMRI food evaluation study.

(A) Plasma glucose. Octreotide significantly increased plasma glucose in patients after RYGB [mean difference (95% CI) 2.2 (0.9, 3.5) mmol/L, F(1,16)=12.78, P=0.003, Cohen's d=2.31], but not in patients after BAND [0.5 (-0.8, 1.8) mmol/L, F(1,16)=0.55, P=0.47, Cohen's d=0.48]. To convert mmol/L to mg/dL multiply by 18.01.

(B) Serum insulin. Octreotide did not have a significant effect on serum insulin in patients after RYGB [mean difference (95% CI) –1.6 (-5.1, 1.9) mU/L, F(1,16)=0.94, P=0.35, Cohen's d=0.26], or in patients after BAND [-0.2 (-3.7, 3.3) mU/L, F(1,16)=0.01, P=0.92, Cohen's d=0.11]. To convert mU/L to pmol/L multiply by 7.175.

(C) Plasma glucagon-like peptide-1 (GLP-1). Octreotide significantly decreased plasma total GLP-1 in patients after RYGB [mean difference (95% CI) -22.6 (-27.3, -17.8) pmol/L, F(1,16)=102.16, P<0.001, Cohen's d=1.80], with a significantly smaller decrease in patients after BAND [-10.4 (-15.1, -5.7) pmol/L, F(1,16)=21.74, P<0.001, Cohen's d=1.90].

(D) Plasma PYY. Octreotide significantly decreased plasma total PYY in patients after RYGB [mean difference (95% CI) -146.8 (-203.5, -90.1) pg/mL, F(1,16)=30.12, P<0.001, d=0.95], with a significantly smaller decrease in patients after BAND [-57.6 (-114.3, -0.88) pg/mL, F(1,16)=4.63, P=0.047, d=1.95]. To convert pg/mL to pmol/L multiply by 0.25.

(E) Plasma fibroblast growth factor-19 (FGF19). Octreotide significantly decreased plasma FGF19 in patients after RYGB [mean difference (95% CI) -28.5 (-50.4, -6.5) pg/mL, F(1,16)=7.54, P=0.014, Cohen's d=0.40], but not in patients after BAND [-12.1 (-36.1, 11.9) pg/mL, F(1,16)=0.02, P=0.90, Cohen's d=0.08].

Data represents mean ± SEM, n=9 per group. Saline (white bars), Octreotide (black bars). *P<0.05, ***P<0.001 with post hoc Fisher's LSD test. For statistics of repeated measures ANOVA for effects of Group, Treatment and interaction see Supplementary Table 4.

Figure 5. Effect of Octreotide on hedonic and brain reward system responses in fMRI food evaluation task after Roux-en-Y gastric bypass (RYGB) and adjustable gastric banding (BAND). **(A,B)** Octreotide increased food appeal ratings in patients after (A) RYGB [mean difference (95% CI) 0.35 (0.01, 0.70), F(1,8)=5.48, P=0.047, Cohen's d=0.43], but not (B) BAND [mean difference (95% CI) 0.049 (-0.38, 0.48), F(1,8)=0.07, P=0.80, Cohen's d=0.07]. **(C,D)** Octreotide significantly increased average BOLD signal (%) to any food picture averaged across all four fROIs (nucleus accumbens, caudate, anterior insula, amygdala) in patients after (C) RYGB [mean difference (95% CI) 0.062 (0.003, 0.122), F(1,6)=6.54, P=0.043, Cohen's d=0.77], but not (D) BAND [mean difference (95% CI) 0.047 (-0.051, 0.146), F(1,8)=1.21, P=0.30, Cohen's d=0.68]. **(E,F)** Octreotide significantly increased average BOLD signal (%) to any food picture in the nucleus accumbens alone in patients after (E) RYGB [0.102 (0.023, 0.180), F(1,6)=10.11, P=0.019, Cohen's d=0.79], but not (F) BAND [mean difference (95% CI) 0.069 (-0.037, 0.174), F(1,8)=2.27, P=0.17, Cohen's d=0.82]. Analysis from repeated measures ANOVA performed separately in each Group, RYGB (n=7, except food appeal rating where n=9) or BAND (n=9), with Treatment (Octreotide or Saline) and energy density of food pictures (high or low) as within subject factors. There was no significant effect of food picture energy density or energy density x Treatment interaction for any fROI in either group. *P<0.05 Octreotide vs. Saline with post hoc Fisher's LSD test. For statistics of repeated measures ANOVA for effects of Group, Treatment and interaction see Supplementary Table 5.

Figure 6. Greater suppression of plasma PYY and GLP-1 with Octreotide is associated with greater increase in brain reward system response in fMRI food evaluation task after bariatric surgery.

The magnitude of the increase in BOLD signal (%) averaged across the four fROIs (nucleus accumbens, caudate, anterior insula, amygdala) to any food picture (high-energy or low energy vs. objects) with Octreotide (Δ Octreotide - Saline) was positively correlated with the decrease in plasma concentration of (A) peptide YY (PYY) with Octreotide (Δ Saline – Octreotide), with a similar trend for (B) plasma concentrations of glucagon-like peptide 1 (GLP-1), independent of the surgical group.

r represent Pearson correlation coefficient. Open circles represent patients after adjustable gastric banding (BAND, n=9), and filled circles patients after Roux-en-Y gastric bypass (RYGB, n=7). Results for PYY are displayed as $\log_{10}$ scale as data were not normally distributed.

One RYGB subject with a decrease in plasma PYY following Octreotide of 401.3 pg/mL was an outlier (<> 2SD) in these analyses. The relationship between Δ BOLD signal and Δ plasma PYY remained significant when excluding this subject (r(14) = +0.54, P=0.036).

FIGURES

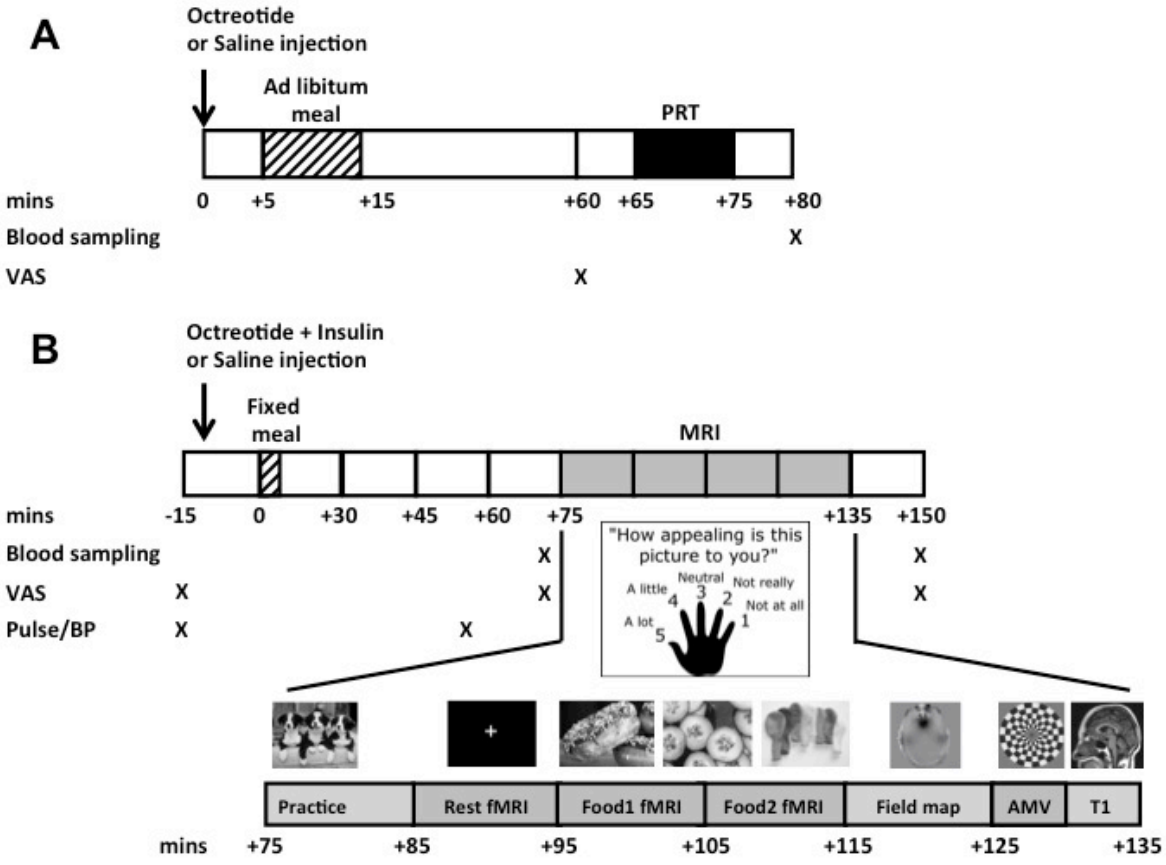


Figure 1

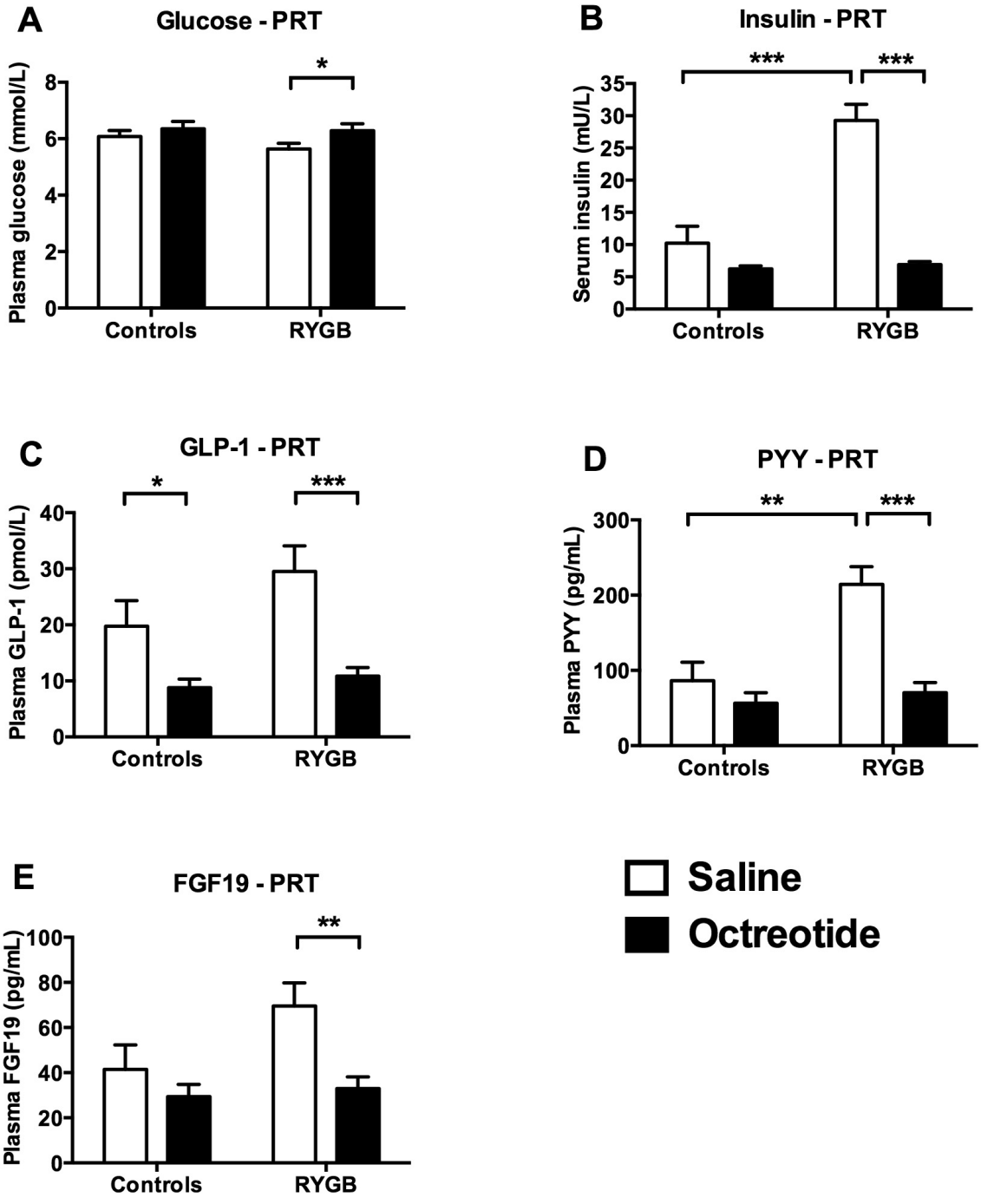

A
Glucose - PRT
Plasma glucose (mmol/L)
0
2
4
6
8
*
Controls
RYGB
B
Insulin - PRT
Serum insulin (mU/L)
0
5
10
15
20
25
30
35
***
***
Controls
RYGB
C
GLP-1 - PRT
Plasma GLP-1 (pmol/L)
0
10
20
30
40
*
***
Controls
RYGB
D
PYY - PRT
Plasma PYY (pg/mL)
0
100
200
300
**
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Controls
RYGB
E
FGF19 - PRT
Plasma FGF19 (pg/mL)
0
20
40
60
80
100
**
Controls
RYGB
Saline
Octreotide

Figure 2

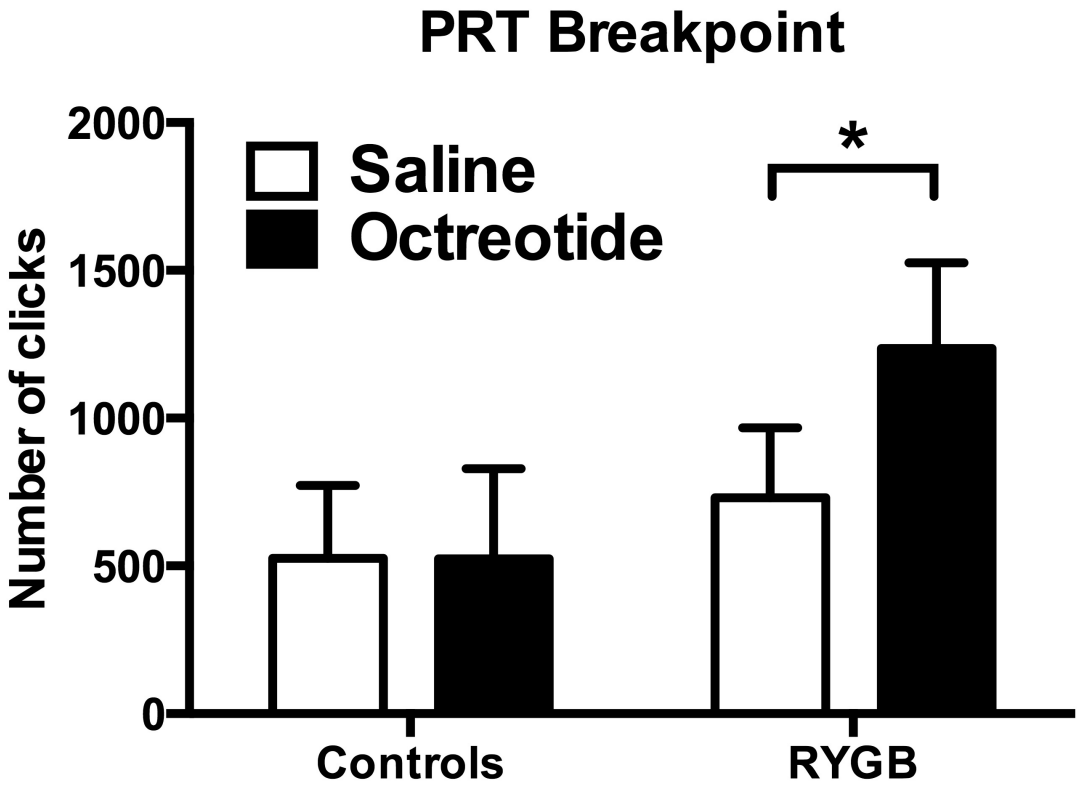

PRT Breakpoint
Number of clicks
2000
1500
1000
500
0
Saline
Octreotide
*
Controls
RYGB

Figure 3

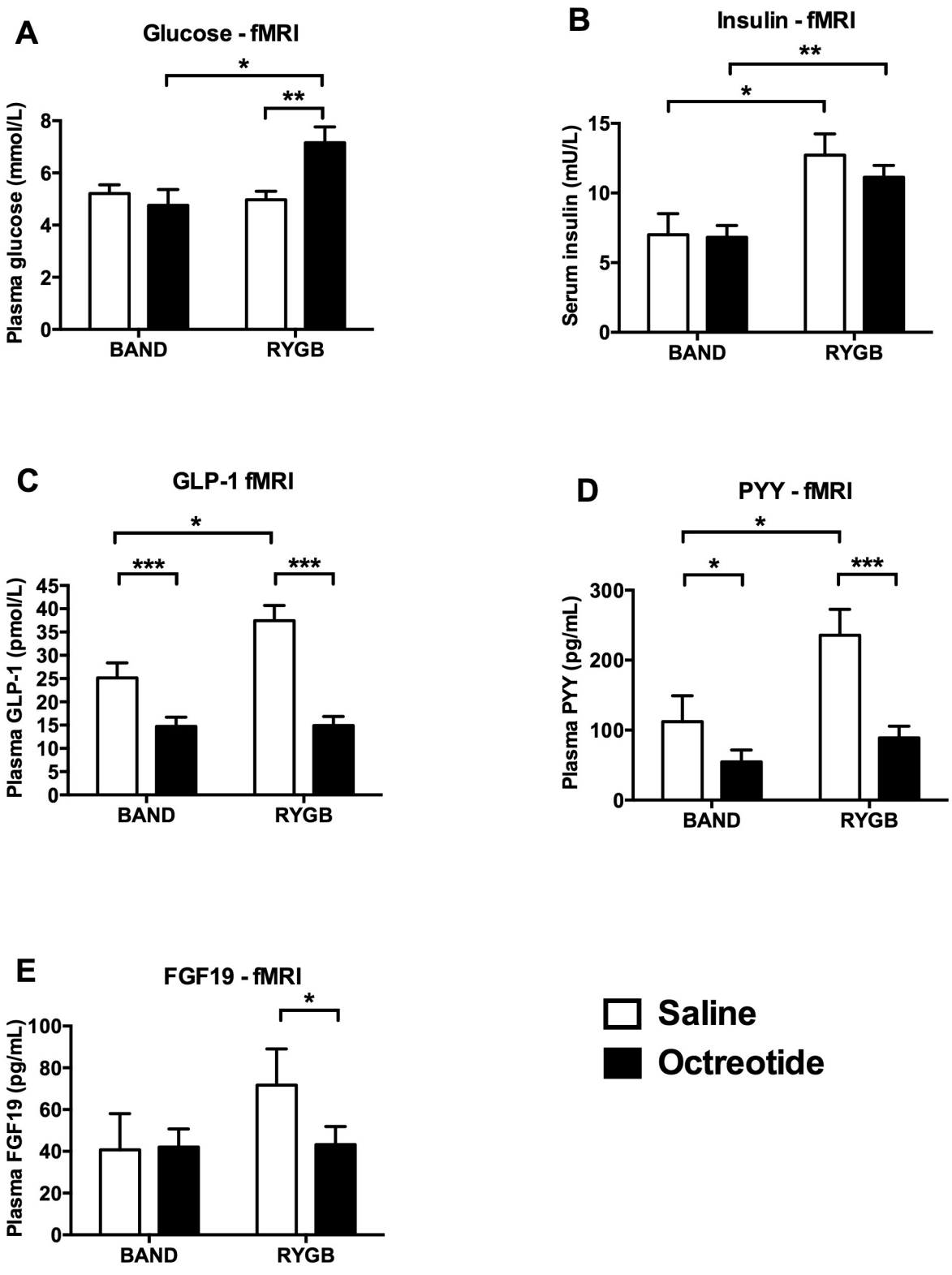

A
Glucose - fMRI
Plasma glucose (mmol/L)
BAND
RYGB
B
Insulin - fMRI
Serum insulin (mU/L)
C
GLP-1 fMRI
Plasma GLP-1 (pmol/L)
D
PYY - fMRI
Plasma PYY (pg/mL)
E
FGF19 - fMRI
Plasma FGF19 (pg/mL)
Saline
Octreotide

Figure 4

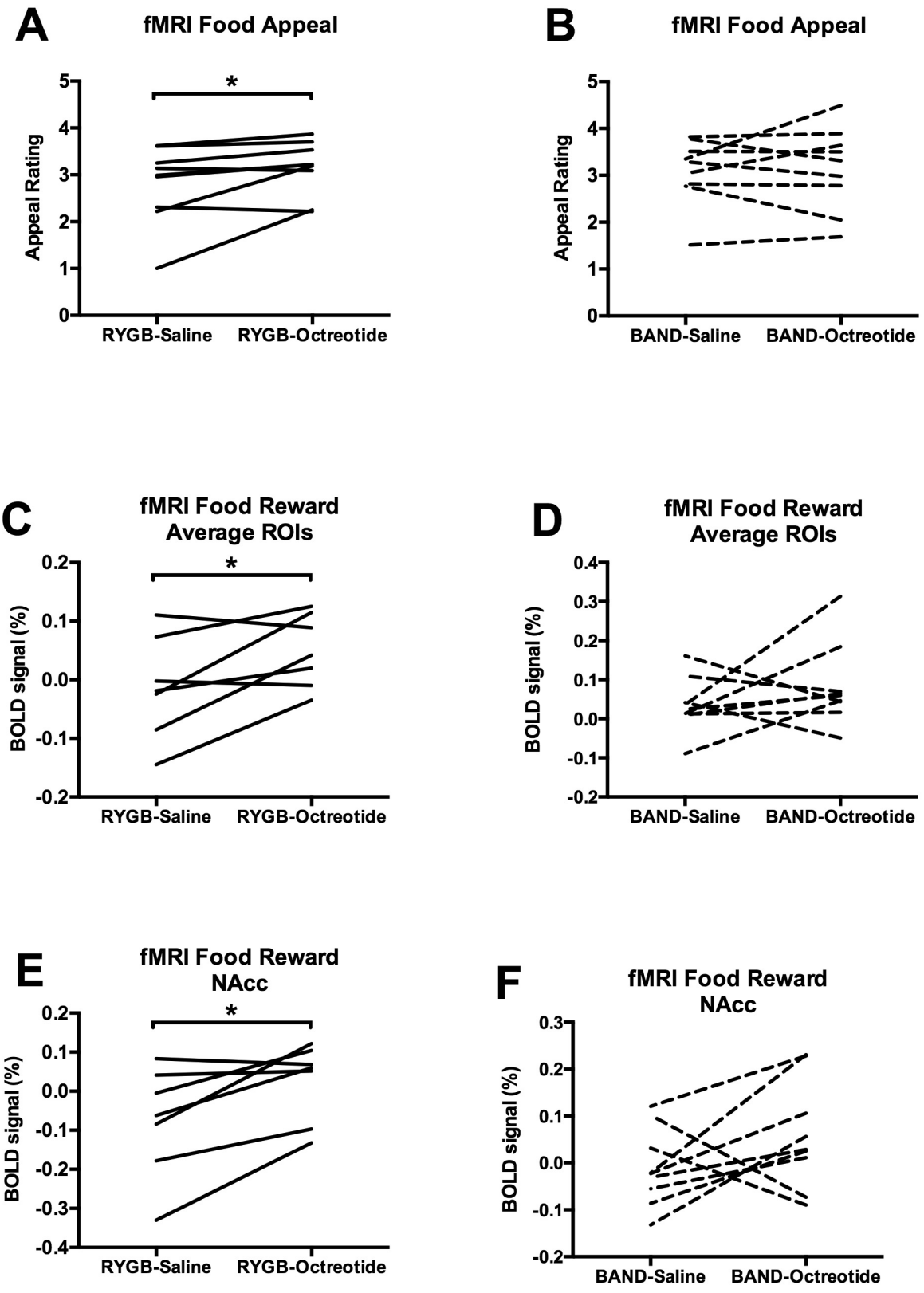

A
fMRI Food Appeal
Appeal Rating
5
4
3
2
1
0
*
RYGB-Saline
RYGB-Octreotide
B
fMRI Food Appeal
Appeal Rating
5
4
3
2
1
0
BAND-Saline
BAND-Octreotide
C
fMRI Food Reward
Average ROIs
BOLD signal (%)
0.2
0.1
0.0
-0.1
-0.2
*
RYGB-Saline
RYGB-Octreotide
D
fMRI Food Reward
Average ROIs
BOLD signal (%)
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
BAND-Saline
BAND-Octreotide
E
fMRI Food Reward
NAcc
BOLD signal (%)
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
*
RYGB-Saline
RYGB-Octreotide
F
fMRI Food Reward
NAcc
BOLD signal (%)
0.3
0.2
0.1
0.0
-0.1
-0.2
BAND-Saline
BAND-Octreotide

Figure 5

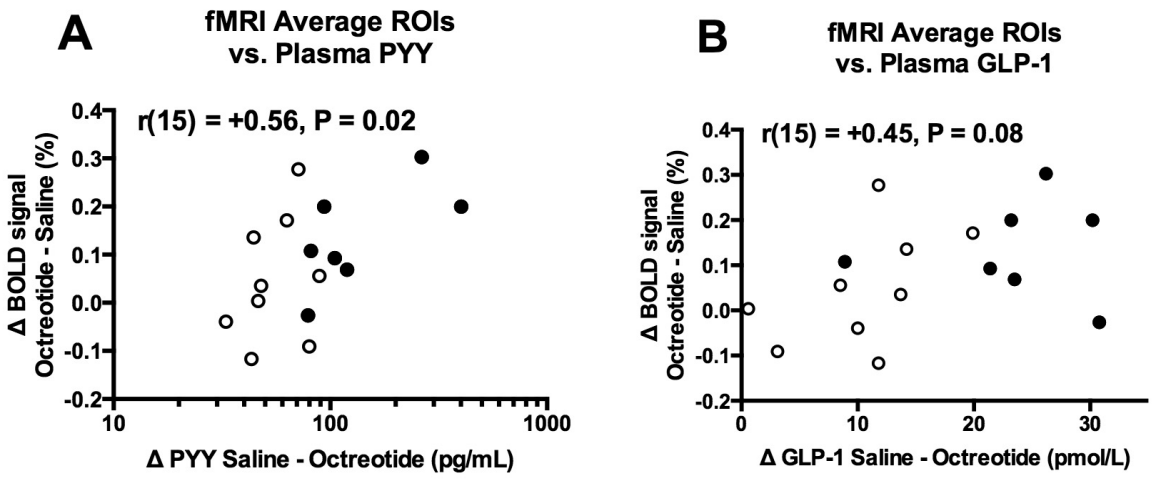

A
fMRI Average ROIs
vs. Plasma PYY
r(15) = +0.56, P = 0.02
Δ BOLD signal
Octreotide - Saline (%)
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
10
100
1000
Δ PYY Saline - Octreotide (pg/mL)
B
fMRI Average ROIs
vs. Plasma GLP-1
r(15) = +0.45, P = 0.08
Δ BOLD signal
Octreotide - Saline (%)
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0
10
20
30
Δ GLP-1 Saline - Octreotide (pmol/L)

Figure 6

SUPPLEMENTARY INFORMATION

Link between increased satiety gut hormones and reduced food reward following gastric bypass surgery for obesity

Goldstone AP, Miras AD, Scholtz S, Jackson S, Neff KJ, Pénicaud L, Geoghegan J, Chhina N, Durighel G, Bell JD, Meillon S, le Roux CW

Supplementary Figures

Supplementary Figure 1. Octreotide increased clicks in the last completed ratio during progressive ratio task after RYGB.

Supplementary Figure 2. Effect of Octreotide on hedonic and brain reward system responses in fMRI food evaluation task after Roux-en-Y gastric bypass (RYGB) and adjustable gastric banding (BAND).

Supplementary Figure 3. Increases of hedonic and brain reward system responses in fMRI food evaluation task with Octreotide after RYGB were greater if lower baseline value.

Supplementary Tables

Supplementary Table 1. Appetite visual analogue scale ratings in progressive ratio task study.

Supplementary Table 2. Effect of Octreotide on plasma glucose and hormones in progressive ratio task study.

Supplementary Table 3. Correlations between change in plasma hormones, and change in breakpoint (total number of clicks) induced by Octreotide in the progressive ratio task study for whole group [controls and Roux-en-Y gastric bypass (RYGB) patients] and RYGB group only.

Supplementary Table 4. Effect of Octreotide on post-prandial plasma glucose and hormones in fMRI food evaluation study.

Supplementary Table 5. Effect of Octreotide on hedonic and brain reward system responses in fMRI food evaluation task after Roux-en-Y gastric bypass (RYGB) and adjustable gastric banding (BAND).

Supplementary Table 6. Appetite visual analogue scale ratings of Roux-en-Y gastric bypass (RYGB) group in fMRI food evaluation study.

Supplementary Table 7. Appetite visual analogue scale ratings of adjustable gastric banding group (BAND) in fMRI food evaluation study.

Supplementary Table 8. Potential confounding variables of Roux-en-Y gastric bypass (RYGB) group in fMRI food evaluation study.

Supplementary Table 9. Potential confounding variables of adjustable gastric banding group (BAND) in fMRI food evaluation study.
Supplementary Table 10. Dumping syndrome signs and symptoms of Roux-en-Y gastric bypass (RYGB) group in fMRI food evaluation study.
Supplementary Table 11. Dumping syndrome signs and symptoms of adjustable gastric banding group (BAND) in fMRI food evaluation study.

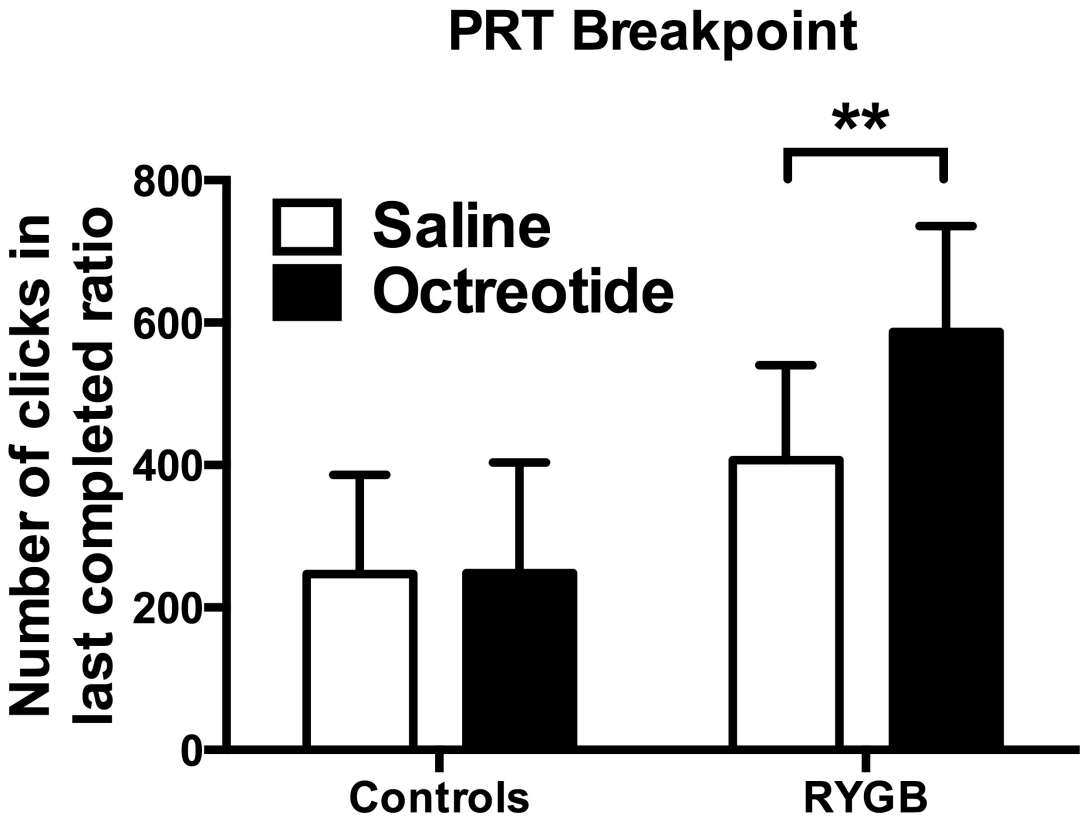
Supplementary Methods

Progressive Ratio Task
Functional MRI Study
Visual Analogue Scales
Glucose and Hormone Assays
Power Calculations

Supplementary References

SUPPLEMENTARY FIGURES

Supplementary Figure 1. Octreotide increased clicks in the last completed ratio during progressive ratio task after RYGB.

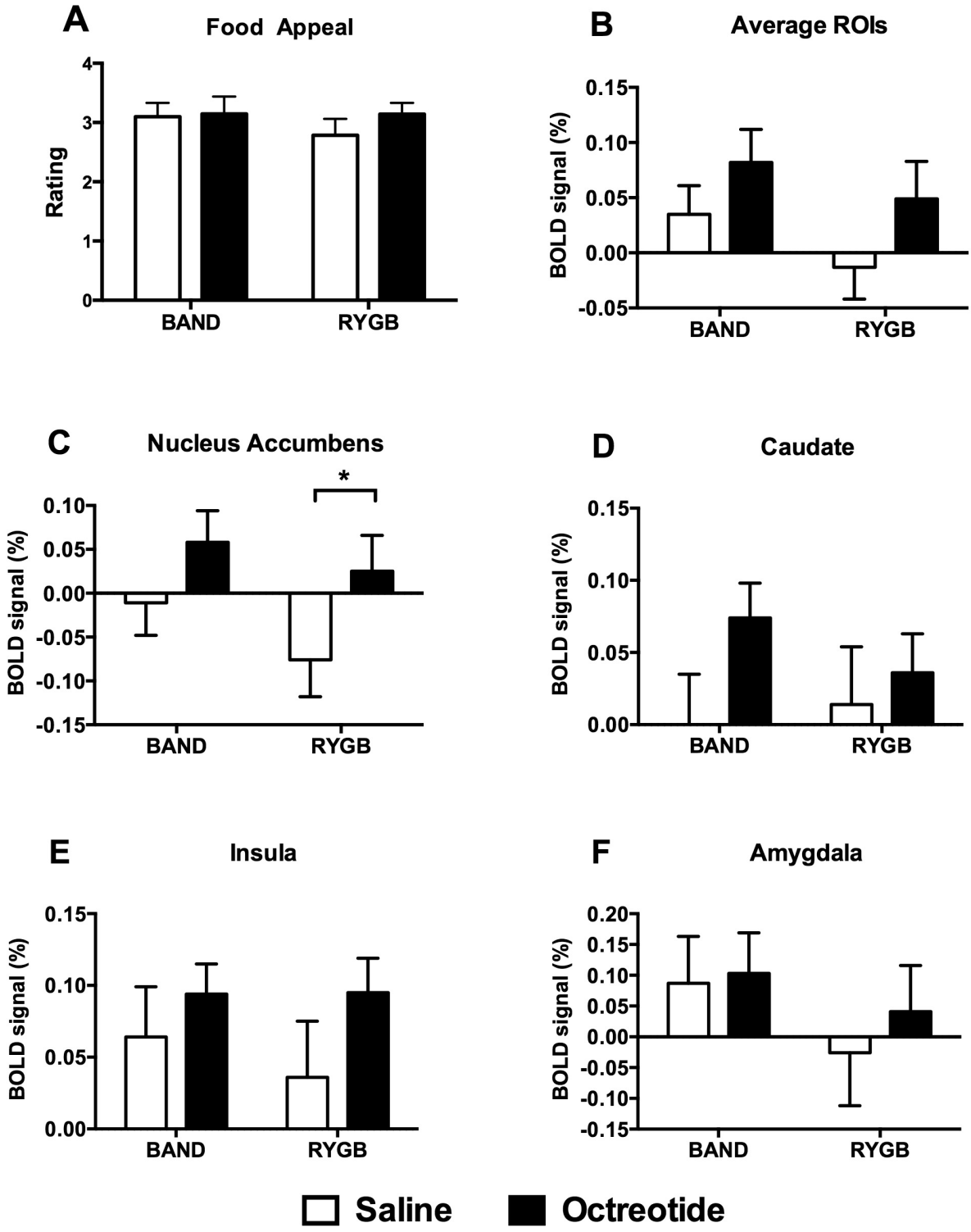


Octreotide significantly increased the number of clicks in the last completed ratio (breakpoint) in patients after RYGB [mean difference (95% CI) 180 (52, 308), F(1,19)=8.73, P=0.008, Cohen's d=0.37], but not in the control group [effect size 1 (-133, 134), F(1,19)=0.00, P=0.99, Cohen's d=0.00]. Overall repeated measures ANOVA: Group F(1,19)=1.57, P=0.23; Treatment F(1,19)=4.20, P=0.54; Group x Treatment F(1,19)=4.11, P=0.057. *P<0.05 with post-hoc Fisher's least significant difference (LSD) test.

Data represents mean ± SEM, n=10 in control and n=11 in RYGB group.

These results are similar to when using the total number of clicks as the measure of the breakpoint (Figure 3).

Supplementary Figure 2. Effect of Octreotide on hedonic and brain reward system responses in fMRI food evaluation task after Roux-en-Y gastric bypass (RYGB) and adjustable gastric banding (BAND)



(A) Food appeal ratings. There was a trend for Octreotide to increase appeal ratings to any food picture in patients after RYGB [mean difference (95% CI) 0.35 (-0.01, 0.71), F(1,16)=4.36, P=0.053, Cohen's d=0.46], but not after BAND [0.049 (-0.311, 0.401), F(1,16)=0.08, P=0.77,

Cohen's d=0.06].

(B) Average reward system fROIs. Octreotide did not have a significant effect on BOLD signal (%) to any food picture across all 4 fROIs in patients after RYGB [mean difference (95% CI) 0.062 (-0.148, 0.023), F(1,14)=2.44, P=0.14, Cohen's d=0.79], or BAND [0.047 (-0.029, 0.123), F(1,14)=1.78, P=0.20, Cohen's d=0.60].

(C) Nucleus accumbens. Octreotide significantly increased BOLD signal (%) to any food picture in the nucleus accumbens in patients after RYGB [mean difference (95% CI) 0.102 (0.006, 0.197), F(1,14)=5.25, P=0.038, Cohen's d=0.92], but not BAND [0.069 (-0.015, 0.153), F(1,14)=3.09, P=0.10, Cohen's d=0.62].

(D) Caudate. Octreotide did not have a significant effect on BOLD signal (%) to any food picture in the caudate nucleus in patients after RYGB [mean difference (95% CI) 0.022 (-0.089, 0.133), F(1,14)=0.19, P=0.67, Cohen's d=0.21], or BAND [0.074 (-0.024, 0.172), F(1,14)=2.62, P=0.13, Cohen's d=0.70].

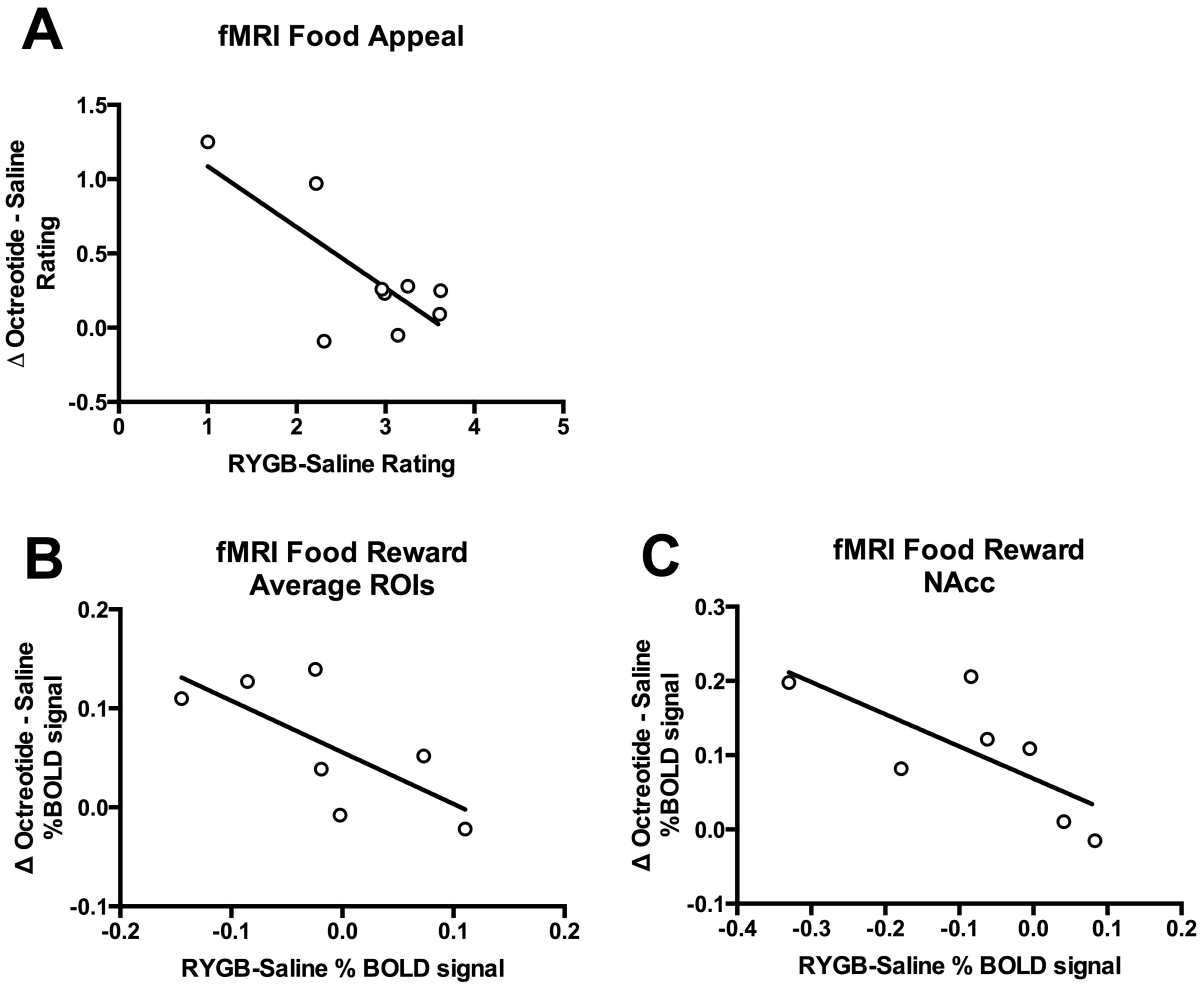
(E) Anterior Insula. Octreotide did not have a significant effect on BOLD signal (%) to any food picture in the anterior insula in patients after RYGB [mean difference (95% CI) 0.059 (-0.044, 0.161), F(1,14)=1.51, P=0.24, Cohen's d=0.57], or BAND [0.031 (-0.059, 0.121), F(1,14)=0.53, P=0.48, Cohen's d=0.30].

(F) Amydgala. Octreotide did not have a significant effect on BOLD signal (%) to any food picture in the amygdala in patients after RYGB [mean difference (95% CI) 0.067 (-0.125, 0.260), F(1,14)=0.57, P=0.47, Cohen's d=0.29], or BAND [0.015 (-0.155, 0.185), F(1,14)=0.04, P=0.85, Cohen's d=0.07].

P values represent post-hoc Fisher LSD for repeated measures ANOVA with Group as between subject factor (RYGB [n=7, except food appeal ratings where n=9] or BAND [n=9]), with Treatment (Octreotide or Saline) and Energy density of food pictures (High or Low) as within subject factors.

Data represents mean ± SEM, n=9 per group. Saline (white bars), Octreotide (black bars). *P<0.05, with post hoc Fisher's LSD test. For F and P values from repeated measures ANOVA for effects of Group, Treatment and Group x Treatment interaction see Supplementary Table 5.

Supplementary Figure 3. Increases of hedonic and brain reward system responses in fMRI food evaluation task with Octreotide after RYGB were greater if lower baseline value.



The magnitude of the increase in food picture appeal ratings with Octreotide (Δ Octreotide - Saline) was negatively correlated with (A) the appeal rating at the Saline visit, Pearson correlation coefficient r(8)=-0.77, P=0.04.

There were similar trends for the magnitude of the increase in (B) BOLD signal averaged across all four fROIs and (C) the NAcc only to negatively correlate with BOLD signal at the Saline visit (r(6)=-0.70, P=0.08; r(6)=-0.72, P=0.07 respectively).

r represents Pearson correlation coefficient.

SUPPLEMENTARY TABLES

Supplementary Table 1. Appetite visual analogue scale ratings in progressive ratio task study.

	Controls		**RYGB**		**ANOVA**					
	Saline	**Octreotide**	**Saline**	**Octreotide**	**Group**		Treatment		**Group x** Treatment	
N	10	10	11	11	F [a]	P [b]	F [a]	P [b]	F [a]	P [b]
Hunger (cm)	1.6 ± 0.6 (0.0, 6.1)	2.0 ± 0.5 (0.2, 4.5)	1.6 ± 0.5 (0.1, 4.9)	0.9 ± 0.2 (0.1, 2.0)	1.36	0.26	0.12	0.73	1.21	0.29
Fullness (cm)	5.7 ± 0.7 (1.0, 8.4)	5.5 ± 0.8 (0.2, 8.4)	6.5 ± 0.8 (0.3, 9.5)	7.2 ± 0.9 (0.5, 10)	1.37	0.26	0.40	0.54	1.07	0.32
Desire to eat (cm)	1.9 ± 0.6 (0.1, 6.4)	2.4 ± 0.5 (0.1, 4.7)	1.1 ± 0.4 (0.1, 4.1)	1.4 ± 0.6 (0.0, 6.3)	3.47	0.08	1.00	0.33	0.00	0.96
Nausea (cm)	0.8 ± 0.6 (0.0, 5.7)	1.0 ± 0.7 (0.0, 6.6)	0.7 ± 0.4 (0.0, 5.1)	1.0 ± 0.5 (0.0, 5.1)	0.25	0.62	0.76	0.39	025	0.62

Data presented as mean ± SEM and (minimum, maximum), with maximum rating 10 cm.

[a] F(1,18) for all ANOVA results.

[b] P values for repeated measures ANOVA with Group as between subject factor (RYGB or Control) and Treatment as within subject factor (Octreotide or Saline).

Abbreviations: RYGB: gastric bypass.

Supplementary Table 2. Effect of Octreotide on plasma glucose and hormones in progressive ratio task study.

	Group		Treatment		Group x Treatment	
	F [a]	P [b]	F [a]	P [b]	F [a]	P [b]
Glucose	0.84	0.37	7.20	**0.013**	1.26	0.28
Insulin	29.10	**<0.001**	49.46	**<0.001**	24.10	**<0.001**
GLP-1	2.49	0.13	23.71	**<0.001**	1.60	0.22
PYY	10.60	0.004	25.77	**<0.001**	11.07	**0.004**
FGF19	3.26	0.09	9.47	**0.006**	2.39	0.14

[a] F(1,19) for all ANOVA results, apart from GLP-1 where F(1,18) as one RYGB subject missing data.

[b] P values for repeated measures ANOVA with Group as between subject factor (RYGB or Control) and Treatment as within subject factor (Octreotide or Saline).

Abbreviations: FGF: fibroblast growth factor, GLP-1: glucagon-like peptide-1, PYY: peptide tyrosine tyrosine, RYGB: gastric bypass.

Supplementary Table 3. Correlations between change in plasma hormones, and change in breakpoint (total number of clicks) induced by Octreotide in the progressive ratio task study for the whole group [controls and Roux-en-Y gastric bypass (RYGB) patients] and RYGB group only.

	Whole group		RYGB group only	
	r [a]	P	r [a]	P
GLP-1	-0.23	0.33	-0.04	0.91
PYY	-0.10	0.67	0.38	0.25
FGF-19	-0.28	0.23	-0.15	0.67

[a] r represents Pearson correlation coefficient of *change* in hormone concentration (average +70 and +150 min time points) and the *change* in breakpoint between the Saline and Octreotide study visits (both variables Δ Octreotide – Saline).

Abbreviations: FGF: fibroblast growth factor, GLP-1: glucagon-like peptide-1, PYY: peptide tyrosine tyrosine, RYGB: gastric bypass.

Supplementary Table 4. Effect of Octreotide on post-prandial plasma glucose and hormones in fMRI food evaluation study.

	Group		Treatment		Group x Treatment	
	F [a]	P [b]	F [a]	P [b]	F [a]	P [b]
Glucose	4.00	0.063	4.01	0.062	9.33	**0.008**
Insulin	14.89	**0.001**	0.58	0.46	0.37	0.55
GLP-1	3.28	0.089	109.08	**<0.001**	14.82	**0.001**
PYY	4.80	**0.044**	29.19	**<0.001**	5.56	**0.031**
FGF19	0.81	0.38	3.42	0.083	4.14	0.059

[a] F(1,16) for all ANOVA results

[b] P values for repeated measures ANOVA with Group as between subject factor (RYGB or BAND) and Treatment as within subject factor (Octreotide or Saline).

Abbreviations: BAND: gastric banding, FGF: fibroblast growth factor, GLP-1: glucagon-like peptide-1, PYY: peptide tyrosine tyrosine, RYGB: gastric bypass.

Supplementary Table 5. Effect of Octreotide on hedonic and brain reward system responses in fMRI food evaluation task after Roux-en-Y gastric bypass (RYGB) and adjustable gastric banding (BAND).

ROI	Group		Treatment		Group x Treatment	
	F [a]	P [b]	F [a]	P [b]	F [a]	P [b]
Food appeal ratings	0.22	0.64	2.82	0.11	1.62	0.22
Average fROIs	1.54	0.24	4.23	0.059	0.08	0.78
NAcc	1.12	0.31	8.30	**0.012**	0.31	0.59
Caudate	0.17	0.69	1.95	0.19	0.56	0.47
Insula	0.21	0.65	1.97	0.18	0.19	0.67
Amygdala	0.97	0.34	0.48	0.50	0.19	0.67

[a] F(1,14) for all ANOVA results, except food appeal rating where F(1,16).

[b] P values for repeated measures ANOVA with Group as between subject factor (RYGB [n=7, except food appeal rating where n=9] or BAND [n=9]), with Treatment (Octreotide or Saline) and Energy density of food pictures (High or Low) as within subject factors. Energy density x Group x Treatment, Energy density x Group, Energy density x Treatment interactions were not significant for any variable (P>0.05).

Abbreviations: fROI: functional region of interest, NAcc: nucleus accumbens.

In the combined surgical group analysis (n=16), Octreotide significantly increased BOLD signal in the nucleus accumbens to any food picture independent of surgical group (mean difference ± SEM 0.085 ± 0.030 (95% CI 0.022, 0.149), Cohen's d 0.76), with a similar trend seen for the BOLD signal averaged across all *a priori* fROIs (mean difference ± SEM 0.055 ± 0.027 (95% CI -0.002, 0.112), Cohen's d 0.69).

Supplementary Table 6. Appetite visual analogue scale ratings of Roux-en-Y gastric bypass (RYGB) group in fMRI food evaluation study.

	Saline	Octreotide	P [a]
n	9	9	
Hunger over fMRI (cm)	1.6 ± 0.5 (0.0, 4.0)	1.8 ± 0.5 (0.0, 4.8)	0.53
Pleasantness to eat over fMRI (cm)	1.8 ± 0.5 (0.0, 5.0)	2.3 ± 0.7 (0.0, 5.7)	0.36
Volume over fMRI (cm)	2.0 ± 0.5 (0.1, 4.9)	1.9 ± 0.6 (0.0, 4.6)	0.88
Fullness over fMRI (cm)	5.7 ± 1.2 (0.7, 9.7)	5.1 ± 1.1 (0.7, 9.2)	0.18

Data presented as mean ± SEM and (minimum, maximum) of average of ratings at +70 and +150 min after breakfast covering fMRI scan, maximum rating 10 cm.

[a] P value for comparison of visits using 2-tailed Student's paired t-test

Supplementary Table 7. Appetite visual analogue scale ratings of adjustable gastric banding group (BAND) in fMRI food evaluation study.

	Saline	Octreotide	P [a]
n	9	9	
Hunger over fMRI (cm)	1.9 [0.3, 3.0] (0.0, 5.1)	1.9 [0.8, 3.3] (0.2, 5.5)	0.95
Pleasantness to eat over fMRI (cm)	2.5 ± 0.7 (0.0, 6.1)	2.5 ± 0.7 (0.3, 6.1)	0.98
Volume over fMRI (cm)	2.6 ± 0.7 (0.1, 5.5)	2.2 ± 0.6 (0.2, 5.5)	0.56
Fullness over fMRI (cm)	5.1 ± 1.1 (1.6, 9.5)	5.5 ± 1.0 (1.5, 9.4)	0.66

Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (minimum, maximum) of average of ratings at +70 and +150 min after breakfast covering fMRI scan, maximum rating 10 cm.

[a] P value for comparison of visits using 2-tailed Student's paired t-test.

Supplementary Table 8. Potential confounding variables of Roux-en-Y gastric bypass (RYGB) group in fMRI food evaluation study.

	Saline	Octreotide	P [a]
n [b]	7	7	
PANAS positive (score /50)	33.1 ± 3.1 (14.0, 48.0)	29.8 ± 3.1 (16.0, 47.0)	0.16
PANAS negative (score /50)	12.5 ± 11.5 (10.0, 19.0)	11.0 [10.5 -14.5] (10.0, 16.0)	0.20
Relative motion during food task (mm/TR)	0.13 [0.08, 0.22] (0.08, 0.48)	0.15 [0.07, 0.25] (0.04, 0.25)	0.33
Relative motion during AMV task (mm/TR)	0.10 [0.06, 0.19] (0.06, 0.41)	0.09 [0.09, 0.29] (0.07, 0.29)	0.92
BOLD signal in bilateral posterior STG - auditory fMRI task (%)	0.702 [0.580, 0.787] (0.312, 1.765)	0.859 [0.370, 1.084] (-0.107, 1.152)	0.74
BOLD signal in left precentral gyrus - motor fMRI task (%)	0.448 [-0.080, 0.603] (-0.604, 0.603)	0.123 [-0.018, 0.531] (-0.083, 0.575)	0.50
BOLD signal in bilateral lingual gyri - visual fMRI task (%)	0.536 ± 0.355 (-1.157, 1.958)	0.478 ± 0.111 (0.093. 0.804)	0.89
Stress over fMRI [c] (cm)	0.2 [0.1, 0.4] (0.0, 1.2)	0.2 [0.1, 1.8] (0.0, 2.8)	0.23
Anxiety over fMRI [c] (cm)	0.1 [0.0, 0.7] (0.0, 1.5)	0.1 [0.1, 1.8] (0.0, 2.8)	0.19

Octreotide administration did not alter BOLD signal during the control auditory-motor-visual task in the RYGB group (multivariate analysis of auditory, motor and visual fROIs: effect of Octreotide $F(1,6)=0.14$, $P=0.72$, effect of fROI x Octreotide $F(2,5)=0.03$, $P=0.97$).

Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (minimum, maximum).

[a] P value for comparison of visits using 2-tailed Student's paired t-test, or Wilcoxon signed rank test, where data is not normally distributed.

[b] excluding 2 subjects who had contraindications to MRI scanning.

[c] average of ratings at +70 and +150 min after breakfast covering fMRI scan, maximum rating 10 cm.

Abbreviations: AMV: Auditory-Motor-Visual, PANAS: Positive and Negative Affect Schedule (Killgore & Yurgelun-Todd 2006), TR: repetition time, STG: superior temporal gyrus, VAS: visual analogue scale.

Supplementary Table 9. Potential confounding variables of adjustable gastric banding group (BAND) in fMRI food evaluation study.

	Saline	Octreotide	P [a]
n	9	9	
PANAS positive (score /50)	33.0 ± 2.2 (26.0, 43.0)	32.6 2.8 (24.0, 47.0)	0.79
PANAS negative (score /50)	13.9 ± 1.3 (10.0, 19.0)	12.9 ± 1.4 (10.0, 23.0)	0.40
Relative motion during food task (mm/TR)	0.14 [0.08, 0.18] (0.06, 0.32)	0.11 [0.09, 0.18] (0.06, 0.29)	0.51
Relative motion during AMV task (mm/TR)	0.10 [0.07, 0.32] (0.07, 0.56)	0.10 [0.06, 0.19] (0.06, 0.31)	0.41
BOLD signal in bilateral posterior STG - auditory fMRI task (%)	0.854 [0.494, 1.061] (-0.179, 1.621)	0.704 [0.209, 1.192] (0.039, 1.451)	0.95
BOLD signal in left precentral gyrus - motor fMRI task (%)	0.492 [0.352, 0.832] (-0.127, 1.313)	0.194 [-0.003, 0.317] (0.078, 0.513)	0.09
BOLD signal in bilateral lingual gyri - visual fMRI task (%)	0.794 ± 0.292 (-0.902, 1.920)	0.623 ± 0.257 (-0.404, 2.133)	0.57
Stress over fMRI [b] (cm)	0.0 [0.2, 2.4] (0.1, 8.0)	0.5 [0.2, 2.9] (0.0, 4.1)	0.34
Anxiety over fMRI [b] (cm)	0.5 [0.2, 2.1] (0.0, 2.9)	0.7 [0.2, 3.0] (0.1, 4.4)	1.00

Octreotide administration did not alter BOLD signal during the control auditory-motor-visual task in the BAND group (multivariate analysis: effect of Octreotide F(1,6)=1.86, P=0.21, effect of fROI x Octreotide F(2,5)=1.24, P=0.35).

Furthermore in combined analysis of RYGB and BAND groups there was no significant Group x Octreotide, or Octreotide effect, in either the auditory [F(1,14)=0.07, P=0.80; F(1,14)=0.35, P=0.56], motor [F(1,14)=1.31, P=0.27; F(1,14)=4.11, P=0.06], or visual [F(1,14)=0.06, P=0.81; F(1,14)=0.24, P=0.63] fROIs.

Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (minimum, maximum).

[a] P value for comparison of visits using 2-tailed Student's paired t-test, or Wilcoxon signed rank test, where data is not normally distributed.

[b] average of ratings at +70 and +150 min after breakfast covering fMRI scan, maximum rating 10 cm.

Abbreviations: see Supplementary Table 8.

Supplementary Table 10. Dumping syndrome signs and symptoms of Roux-en-Y gastric bypass (RYGB) group in fMRI food evaluation study.

	Saline	Octreotide	P [a]
N	9	9	
Δ Heart rate after breakfast [b] (beats / min)	12.0 [4.0, 16.0] (-5.0, 35.0)	-1.0 [-2.5, 5.5] (-9.0, 33.0)	0.16
Δ Systolic BP after breakfast [b] (mm Hg)	-8.3 ± 3.0 (-12.0, 20.0)	5.0 ± 4.3 (-18.0, 25.0)	0.47
Δ Diastolic BP after breakfast [b] (mm Hg)	11.2 ± 3.1 (-5.0, 26.0)	1.7 ± 4.5 (-29.0, 16.0)	0.12
Sleepiness over fMRI [c] (cm)	2.2 ± 0.6 (0.1, 5.8)	1.7 ± 0.4 (0.1, 3.4)	0.43
Nausea over fMRI [c] (cm)	0.6 [0.1, 2.5] (0.0, 5.8)	0.2 [0.0, 1.1] (0.0, 3.0)	0.30

There were no significant effects of Octreotide on post-prandial signs and symptoms of dumping syndrome e.g. changes in heart rate or blood pressure, sleepiness, nausea in the RYGB group (Ukleja 2005).

Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (minimum, maximum).

[a] P value for comparison of visits using 2-tailed Student's paired t-test, or Wilcoxon signed rank test where data is not normally distributed.

[b] change between values at time points -15 and +55 min after breakfast (at 0 min).

[c] average of ratings at +70 and +150 min after breakfast covering fMRI scan, maximum 10 cm.

Abbreviations: BP: blood pressure.

Supplementary Table 11. Dumping syndrome signs and symptoms of adjustable gastric banding group (BAND) in fMRI food evaluation study.

	Saline	Octreotide	P [a]
N	9	9	
Δ Heart rate after breakfast [b] (beats / min)	1.8 ± 2.0 (-9.0, 9.0)	-0.2 ± 2.0 (-7.0, 12.0)	0.25
Δ Systolic BP after breakfast [b] (mm Hg)	8.1 ± 3.7 (-11.0, 24.0)	6.0 3.7 (-9.0, 32.0)	0.72
Δ Diastolic BP after breakfast [b] (mm Hg)	10.0 ± 2.6 (-3.0, 20.0)	7.6 ± 4.8 (-15.0, 37.0)	0.68
Sleepiness over fMRI [c] (cm)	3.0 ± 0.9 (0.0, 8.0)	3.4 ± 0.9 (0.1, 7.0)	0.53
Nausea over fMRI [c] (cm)	0.4 [0.2, 2.7] (0.0, 3.9)	1.1 [0.2, 4.0] (0.1, 6.0)	0.35

There were no significant effects of Octreotide on post-prandial signs and symptoms of dumping syndrome e.g. changes in heart rate or blood pressure, sleepiness, nausea in the BAND group (Ukleja 2005).

Data presented as mean ± SEM or median [interquartile range] for data that is not normally distributed, and (minimum, maximum).

[a] P value for comparison of visits using 2-tailed Student's paired t-test, or Wilcoxon signed rank test where data is not normally distributed.

[b] change between values at time points -15 and +55 min after breakfast (at 0 min).

[c] average of ratings at +70 and +150 min after breakfast covering fMRI scan, maximum 10 cm.

Abbreviations: BP: blood pressure.

SUPPLEMENTARY METHODS

All studies were performed in accordance with the principles of the Declaration of Helsinki, and all participants provided written informed consent.

Progressive Ratio Task

The PRT study was approved by St. Vincent University Hospital Ethics Committee [Roux June 12 (1)], and registered at ClinicalTrials.gov (NCT02010385).

The instructions given to participants were as follows: "In the task you can earn M&M by clicking on the mouse button. You need to click on the BLUE panel for your clicks to count. When the message box appears it means that you have earned food. After you have swallowed the M&M you may click on OK to continue with the programme. Click as much or as little as you like. When you no longer want to continue, press the spacebar to stop the session. There are no right or wrong answers. This is not a competition."

Ad libitum breakfast

The presented foods for breakfast were cereals, porridge, toast with jam and/or butter or cheese, fried eggs, orange juice, milk, fruits, croissant, scones, coffee and tea.

Functional MRI Study

The fMRI study was approved by the West London Research Ethics Committee (REC 08/H0707/139).

Participants

Eleven patients who had previously undergone RYGB surgery were recruited from the Imperial Weight Centre, Imperial College Healthcare NHS Trust, Charing Cross Hospital, London. Seven RYGB patients from a previously published fMRI study (Scholtz et al. 2014), re-attended for two further study visits. An additional two subjects had to be excluded from analysis due to insufficient sleep the night before scanning or feeling unwell on the study visit. Two further RYGB subjects completed identical study visits and the food picture evaluation task outside the MRI scanner due to contraindications to MR scanning. Therefore in total there were nine patients after RYGB included in the final analysis, of which two had not participated in the

previous study and only had behavioural data available. Nine patients who had previously undergone BAND surgery from our previous fMRI study were also recruited to re-attend for two further study visits (Scholtz et al. 2014).

Scanning visit protocol

On the day before each scanning visit, subjects were instructed to avoid exercise and alcohol intake, to eat their usual supper at 20:00h, and then attend the Clinical Investigation Unit, Hammersmith Hospital, London, in the morning having eaten or drunk nothing since supper the evening before other than water. Females were scanned in the first 14 days of the menstrual cycle to attenuate variations in reward responses (Frank et al. 2010). The two study visits were at least 6 days apart, with median [interquartile range] between visits 14.0 [7.0, 20.3] days.

Subjects had measurements of height, weight, % body fat by bio-electrical impedance analysis (Bodystat 1500, Isle of Man, UK), and insertion of a venous cannula in the antecubital fossa at least 30 mins before the start of serial blood sampling. Randomisation order used a sequential list of visits. Patients were scanned for 1 hour starting between 11:00 and 12:00h.

A doctor who was not otherwise involved in the study visit prepared the injections the morning of the study and kept the key to randomisation; the study visit was conducted by investigators who were therefore blinded to the contents of the syringes.

Functional regions of interest

BOLD signal for the contrasts high-energy food > object and low-energy food > object were measured in the following functional regions of interest (fROIs): amygdala, nucleus accumbens, anterior insula and caudate nucleus (Scholtz et al. 2014; Goldstone et al. 2014). We were unable to include the OFC as a fROI as in our previous study (Scholtz et al. 2014), as a result of excessive T2* signal drop out in this region due to the recognized artefact from frontal sinuses, despite use of field map correction and angled slice acquisition (Deichmann et al. 2003).

The average (median) magnitude of bilateral BOLD signal within each *a priori* fROI was extracted for each individual subject separately for the high-energy food > object and low-energy food > object contrasts using featquery software in FSL.

Confounding variables

At each fMRI study visit, positive and negative affect over the preceding week (Positive and Negative Affect Schedule, PANAS) (Killgore & Yurgelun-Todd 2006), head motion during fMRI tasks, anxiety and stress VAS ratings, were also measured, as confounding factors that could influence BOLD signal.

Visual Analogue Scales

The questions asked (and anchors) for visual VAS ratings were as follows: "How hungry/full/sick/sleepy/anxious/stressed do you feel right now?" (Not at all, Extremely); "How pleasant would it be to eat right now?" (Not at all, Extremely); "How much do you think you could eat right now?" (Nothing, A large amount).

Glucose and Hormone Assays

Blood samples for gut hormone and FGF19 analysis were collected into chilled lithium heparin polypropylene tubes, containing 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) (A8456 Sigma-Aldrich) (fMRI study only) and aprotinin (Nordic Phama UK) protease inhibitor (both studies) to give final concentration of 1 mg/mL and 200 kIU/mL whole blood respectively. Blood samples were centrifuged at 4^{o}C, 4000 rpm for 10 min. Aliquots of separated plasma were stored at -80°C until assay.

Plasma glucose was measured using either an Abbott Architect ci8200 analyzer (Abbott Diagnostics, Maidenhead, UK) or an Axsym analyzer (Abbott Diagnostics, Maidenhead, UK). Intra-assay coefficients of variation of all measurements were 1.0-5.0%. Insulin was measured using automated methods (Roche, UK) with an intra-assay coefficient of variation of 4.5%. Plasma GLP-1 was measured in duplicate by ELISA (Linco, UK). This assay measures total GLP-1 (GLP-$1_{7\text{-}36\ amide}$ and GLP-$1_{9\text{-}36\ amide}$) with an intra-assay CV of 5.1%. Plasma PYY was measured in duplicate by an ELISA (Linco, UK). The assay measured total PYY ($PYY_{1\text{-}36}$ and $PYY_{3\text{-}36}$) with a CV of 7.5%. Plasma FGF19 concentration was measured using a quantitative sandwich ELISA technique (FGF19 Quantikine ELISA kit, catalogue no. DF1900; R&D Systems, Minneapolis, MN).

Power Calculations

Power calculations were performed for the fMRI food evaluation task using data from the RYGB group to examine if we were underpowered to detect changes with Octreotide of a similar

magnitude in the BAND group. With n=9 and alpha=0.05, we would have power of 1.0 to detect a mean difference in food appeal rating of 0.354 with SD 0.141, power of 0.74 to detect a mean difference in average % BOLD signal across all four fROIs of 0.062 with SD 0.024, and power of 0.88 to detect a mean difference in % BOLD signal in nucleus accumbens of 0.102 with SD 0.085.

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