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Effects of leptin deficiency and replacement on cerebellar response to food-related cues

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Abstract

Leptin affects eating behavior partly by altering the response of the brain to food-related stimuli. Effects of leptin on brain structure have been observed in the cerebellum, where leptin receptors are most densely expressed, but the function of leptin in the cerebellum remains unclear. We performed a nonrandomized, prospective interventional study of three adults with genetically-mediated leptin deficiency. fMRI was recorded three times each year during years 5 and 6 of leptin replacement treatment. Session one of each year occurred after 10 months of continuous daily replacement, session two after 33–37 days without leptin, and session three 14–23 days after daily replacement was restored. Statistical parametric mapping software (SPM5) was employed to contrast the fMRI blood-oxygenation level-dependent response to images of high-calorie foods versus images of brick walls. Covariate analyses quantified the effects of the duration of leptin replacement and concomitant changes in body mass on the cerebral responses. Longer duration of replacement was associated with more activation by food images in a ventral portion of the posterior lobe of the cerebellum, while simultaneous decreases in body mass were associated with decreased activation in a more dorsal portion of the same lobe. These findings indicate that leptin replacement reversibly alters neural function within the posterior cerebellum, and modulates plasticity-dependent brain physiology in response to food cues. The results suggest an underexplored role for the posterior cerebellum in the regulation of leptin-mediated processes related to food intake.

Keywords

obesity; leptin; fMRI; plasticity

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Introduction

Two-thirds of the adult population of the United States is overweight or obese [1, 2]. As this epidemic had been recognized since the 1980s, the discovery, over a decade ago, of the adipocyte-synthesized, satiety hormone leptin and its ability to modulate body weight in mice generated great interest [3]. We have shown that daily leptin replacement to three obese adults who were congenitally leptin-deficient, due to a rare mutation in the obese(*ob*) gene [4], normalized endocrine function and eating behavior [5]. While body weight decreased during the initial 18 months of leptin replacement, grey matter (GM) concentration increased in the cerebellum, anterior cingulate gyrus, and the inferior parietal lobule [6]. This observation suggested that effects of leptin on behavior may be mediated, in part, by plastic structure-function brain remodeling.

Later, leptin supplementation to the same adults was withheld for several weeks each year in 2005, 2006 and 2007 (years 4, 5 and 6, respectively, after the initiation of treatment). In years 5 and 6, the BOLD (Blood-Oxygen-Level Dependent) whole-brain fMRI response to visual food-related stimuli was assessed. In year 5, we showed that withholding leptin altered brain response to images of high-calorie foods, and increased both hunger ratings elicited by these images, and the patients' body mass index (BMI) [7]. Most recently, we reported that the local GM increases documented during the initial 18 months of leptin replacement [6] were partially or completely reversed during the periods when leptin was withheld [8]. The structural effects in brain were associated both with the duration of leptin replacement and with the changes in BMI.

The analysis presented here was done to determine whether the functional response to food-related cues in the brain was reversible, and if it showed plastic changes at the same locations in which structural brain scans revealed changes in tissue composition. The effects of leptin on BOLD signal might, to some extent, reflect structural alterations. Therefore, the fMRI results from year 5 and previously unreported fMRI results from year 6 were combined to assess the effects of duration of leptin replacement and BMI on the cerebral response to food-related cues, and to explore how these effects might be related to the structural effects of leptin in these patients [6, 8].

The cerebellum was of particular interest for several reasons. Structural effects of initial leptin administration in these patients both after six and eighteen months [6], and their reversal by withholding leptin supplementation [8] achieved the largest t-values in the cerebellum. In our initial study of cerebral response to high-calorie food cues in these patients, most voxels where leptin was associated with activation were also in the cerebellum [7]. Although the cerebellum has been primarily associated with motor functions, and the pro-satiety effects of leptin are thought to be primarily hypothalamic, leptin receptors in the brain, particularly the OB-Rb long form of the receptor which is widely considered to be the signaling-competent isoform [9], are most densely expressed in the cerebellum. Moreover, leptin exerts potent neuritogenic and anti-apoptotic effects on specific cerebellar neuronal populations [10], and a recent reconceptualization has implicated cerebellar function in both cognitive and emotional processing [11–13].

Materials & Methods

Patients and leptin administration

Three leptin-deficient adult patients participated in this study, which was approved by the Food and Drug Administration and by the UCLA Institutional Review Board. Informed, written consent was given by all subjects. Initial leptin doses in 2001 were 0.02 to 0.04 mg/kg once a day at 6:00 pm, designed to achieve a normal leptin concentration based on a body

fat of 30% in females and 20% in males. The doses were later decreased as the patients lost weight, in order to avoid excessively rapid weight loss. Then each of the patients was maintained on the same dose for the duration of the study (patient A: 30-yr-old male = 0.15 mg/day; patient B: 39-yr-old female = 0.2 mg/day; patient C: 44-yr-old female = 2.5 mg/day). Higher doses in patient C were required to normalize metabolic and endocrine parameters because she suffers from common obesity, and therefore has some degree of leptin resistance [14].

The clinical response to the initial leptin replacement has been described extensively [4, 5, 15–22]. In brief, leptin replacement led to resolution of hyperphagia and to massive weight loss [23]. The mean BMI ($\pm$ sd) decreased from 51.2 ± 2.5 kg/m² at baseline to 36.5 ± 2.3 kg/m² after 6 months; BMI was 28.9 ± 3.2 kg/m² after 12 months of treatment and 26.9 ± 2.1 kg/m² after 18 months of treatment. The weight losses of patients A, B, and C after 18 months of treatment were 76.2, 47.5, and 60.0 kg, respectively, corresponding to 53.8%, 43.5%, and 44.5% of their pre-treatment body weights, respectively. After the initial loss, weight stabilized. With weight loss, Patient C, who was diabetic, became normoglycemic. Important metabolic changes, such as decreases in serum free fatty acids, triglycerides, total cholesterol, insulin and insulin resistance, were also observed. The patients had hypogonadotropic hypogonadism, but became eugonadal after treatment. The circadian rhythms of cortisol and TSH also normalized [23].

Data Acquisition

Participants came to UCLA three times in 2006 (year 5) and in 2007 (year 6) for assessment of the cerebral response to high-quality, color photographs in three categories: high-calorie foods (e.g., cheeseburgers, pizza), low-calorie foods (e.g., salad, strawberries), and brick walls (control). Three stimulus sets were prepared, containing eight photographs in each category. T2*-weighted, gradient-recalled, echoplanar images with blood oxygen level-dependent (BOLD) contrast (repetition time, 1500 ms; echo time, 30; flip angle, 70; 26 4-mm axial slices covering the entire brain with 1-mm inter-slice intervals; matrix, 64×64 ; 3.12 mm² in-plane resolution) were collected on a 3-T MRI scanner (Siemens Allegra).

During each MRI session, a set of 240 images of each axial slice were collected during two or three 6-min runs, comprised of twelve 30-sec trials (four per stimulus category). In each trial, participants were presented with two sequentially appearing images of the same category (9 sec each) followed by a visual prompt (12 sec). At the prompt, they were to indicate how hungry the images made them feel by pressing a button from 1 to 7 times (1 press = “Not at all”; 7 presses = “very hungry”). The first assessment in each year (LEPTIN + condition) was scheduled after > 10 months of uninterrupted daily leptin replacement; the second (LEPTIN) after slightly over a month without leptin (32 days in year 5, 37 days in year 6); and the third (LEPTIN brief) 14–23 days after daily leptin supplements were restored (14 days in year 5, 18 – 23 days in year 6). Two sets of stimuli (12 min total duration) were presented during each fMRI session in year 5, and all three sets of stimuli (18 min total duration) were presented in each fMRI session in year 6. On a separate day, but within 72 h of each session, T1-weighted MPRAGE structural MRI images of the whole brain were acquired as previously described [8].

Details of the experimental procedure and preprocessing of the functional images have been published [7]. Briefly, all functional images were aligned to the first functional image, spatially normalized to the atlas space developed at the Montreal Neurological Institute (MNI space), spatially smoothed with a 6-mm full-width half-maximal Gaussian filter, and temporally high-pass filtered at 128-sec with six individual movement parameters applied as covariates of no interest. Table 1 presents the number of days of ongoing leptin replacement and the BMI of each patient at the time of each fMRI session.

Statistical Analysis

The data were analyzed with a statistical parametric mapping software package (SPM version 5 - <http://www.fil.ion.ucl.ac.uk/spm/software/spm5/>). This package computes statistical parametric maps (SPMs) including t-test results for contiguous “clusters” of voxels which pass the threshold alpha level for a given contrast. The threshold alpha level in this study was $p < 0.001$ (without correction for multiple comparisons in whole brain) for the effects of leptin and BMI on the contrast between conditions (food-related stimuli vs. brick walls). Statistical results calculated by SPM5 include probabilities at the level of the set (i.e., the probability of finding a given number of clusters in a given search volume), cluster (i.e., the probability associated with the spatial extent [size] of each cluster), and voxel (i.e., the probability associated with the magnitude of the assessed effect at each voxel).

For each subject, a fixed-effect first-level analysis of all 15 6-min blocks of image data (2 runs x 3 sessions in year 5, 3 runs x 3 sessions in year 6) employed the general linear model. Time-courses were constructed for four conditions (high-calorie foods, low-calorie foods, brick walls and the rating period) by convolving each block with the canonical hemodynamic response function. For each of the three sessions in each of the two years (six images per subject), we generated separate atlas-normalized contrast images (2-mm isotropic voxels) reflecting BOLD response to images of high-calorie foods, minus the response to images of brick walls.

These 18 brain images were then entered into a 2nd-level analysis using a flexible factorial design modeling subject and two covariates; the duration of leptin treatment and BMI of the participant at the time of each scanning session. We recently employed a similar design to assess structural effects of leptin in the same patients, with that analysis also including data collected in year 4 [8].

Our evidential criterion for the analysis was spatial extent with $p < 0.05$ after multiple-comparisons correction for the whole-brain search volume. We also conducted a region-of-interest analysis to determine if leptin affected the neural response to food-related cues in the same three locations where initial leptin replacement increased gray matter [6]. For this analysis, the evidential criterion was spatial extent with $p < 0.05$ for the cluster which contained the suprathreshold voxel closest to the location of the *a priori* hypothesized effect. This is a standard test in the SPM package, which can be accessed at any hypothesized location within a statistical parametric t-map. To constitute evidence, we also required the closest cluster to be within the anatomic structure of interest – the cerebellum, anterior cingulate gyrus or inferior parietal lobule. Finally, a Bonferroni multiple-comparison correction ($0.05/3 = 0.017$) was applied to the test of the superordinate hypothesis that withholding leptin altered functional activity in the same three structures where the initial replacement increased GM.

In order to explore the relative contributions of direct effects of leptin and secondary effects mediated by changes in body mass and fat content, our analysis modeled the covariation of the BOLD response on the day of each scan with two indices: the BMI, which is a direct function of body mass and height, and the number of contiguous days of leptin replacement. A value of 300 days was used for the first scan each year, when leptin replacement had been ongoing for at least 10 months. Negative numbers quantified the number of days since leptin had been stopped at the second scan of each year, and positive numbers quantified the number of days since replacement was restarted at the third scan of each year.

The fMRI analysis for year 5 in our previously published study [7] contrasted the response elicited by pictures of high-calorie foods with those elicited by pictures of low-calorie foods,

and showed effects of leptin in several structures, including those where GM changes had been observed (i.e., the cerebellum, frontomedial cortex and parietal lobe). Here, we combine fMRI data from years 5 and 6, and contrast the response elicited by pictures of high-calorie foods with that elicited by pictures of brick walls. We reasoned that this contrast should be more sensitive to effects of leptin than comparing two categories of foods because leptin deficiency may cause all food-related stimuli to activate brain networks associated with hunger, creating a ceiling effect when comparing two types of food-related pictures. Although contrasting pictures of food with brick walls is not as well-controlled for the physical characteristics of the images, there is good reason to expect leptin replacement to change responses related to hunger but not reactions to physical characteristics of pictures. In addition, differences between the physical characteristics of the pictures of food and brick walls remained constant at the different periods of leptin replacement, but hunger did not.

Results

Changes in Body Mass

Withholding leptin replacement resulted in increased weight and BMI at the second functional scan of each year, as compared to the first scan (see Table 1). The average increase in weight per day without leptin was 0.20 kg for patient A, 0.12 kg for patient B, and 0.17 kg for patient C. During the period of brief replacement between scans 2 and 3, there were trivial and inconsistent changes in weight. Patients A and B lost all the weight gained while leptin was withheld in year 5 during the >10 months of leptin replacement before the first scan in year 6 (see Table 1). Patient C, who suffers from common obesity and leptin resistance, lost only 3% of the weight gained in year 5 before the first scan in year 6, and gained more weight when leptin was withheld in year 6 than in year 5.

Brain areas activated by pictures of high-calorie foods

We performed a t-test against a mean of zero for the contrast images (high-calorie food - brick wall) across all sessions and subjects, to assure that the food-related stimuli were salient and the scanning paradigm valid for the population studied. Using thresholds of $p < 0.001$ with >10 contiguous voxels, there were significant and extensive bilateral activations within cerebellum, occipital cortex, inferior frontal gyrus, insula, thalamus, striatum, midbrain, hippocampus and amygdala (Figure 1).

Covariation with Number of Days of Leptin Replacement

Whole-brain analysis indicated no areas where the BOLD response to pictures of high-calorie foods was inversely related to the number of days of contiguous leptin replacement. BOLD response was, however, directly related to the number of days of contiguous leptin replacement in several locations within the cerebellum (Table 2, top; Figure 2, middle column).

The cluster closest to the cerebellar location where initial leptin replacement increased gray matter concentration [6] included 121 voxels (spatial extent $p < 0.002$ for a hypothesized effect, Table 2). The clusters closest to the expected anterior cingulate and parietal lobe locations [6] were not significant.

Covariation with BMI

Whole-brain analysis indicated no areas where BOLD signal was inversely related to BMI. BOLD signal was, however, directly related to BMI within the cerebellum and parietal lobe (Table 2, bottom; Figure 2, left column). The clusters closest to the three locations where

initial leptin replacement increased gray matter concentration [6] were not significant for spatial extent.

Discussion

In previous fMRI studies, visual food-related stimuli, as compared to non-food stimuli, reliably elicited activation within occipital, insular, and frontal cortices, the striatum, and the amygdala [24–30]. Here, images of desirable foods, as compared to brick walls, elicited bilateral activation in all of these regions and in the following subcortical regions activated in some, but not all prior studies: thalamus, midbrain, hippocampus and posterior cerebellum. Although a few previous studies reported cerebellar activation [25, 27], activation in occipital cortex was consistently reported. Some occipital clusters may have extended into neighboring posterior cerebellum, as seen here, and also in all four patient groups of a recent report which made no mention of the cerebellum (compare our Figure 1 to Figure 1 of [29]). These observations suggest that cerebellar activation by food-related visual stimuli may be underreported.

Visual food-related stimuli activate more of the brain when participants, especially women [31], are hungry than when they are satiated [32, 33]. As images of high-calorie foods elicited more hunger after leptin replacement was withheld than when replacement was ongoing in year 5 of this study [7], we expected the images of food to produce a greater distribution of brain activation when replacement was withheld. When assessing the effects of leptin on brain activation by food pictures that same year, however, about six times as many voxels exhibited greater activation during leptin replacement, when hunger was low, as compared to when replacement was withheld and hunger was high (1288 voxels in Table 4 vs. 219 voxels in Table 3 of [7]). Moreover, 51% of the voxels that were more activated during leptin replacement, including those with the largest effects, were in the cerebellum, where regional cerebral blood flow, a measure of neural activation, is attenuated during satiety in normal-weight men [34], and in both lean and obese women [35].

Our recent finding that withholding leptin reversibly decreased GM concentration in several brain regions of these patients, particularly in the cerebellum [8], offered a possible explanation. BOLD signal may be lower after withholding leptin in an area with less GM signal, possibly indicating reduced neuronal density or size. In addition, the weight and the body fat of the patients increased when leptin was withheld [7], and both obesity and body fat content have been associated with GM deficits in cross-sectional studies [36–39]. Because the contribution of leptin to these associations remains unclear, we previously evaluated effects of withholding leptin on brain structure [8] by employing the two-covariate design (duration of leptin replacement and BMI) used in the present study. Withholding leptin replacement reversed the increases in GM concentration observed within the cerebellum and anterior cingulate gyrus during the first 18 months of leptin replacement. GM changes in the anterior cingulate gyrus were best explained by the changes in BMI, whereas changes in the cerebellum were best explained by the duration of leptin replacement.

In the study reported here, duration of leptin replacement showed direct covariation with brain activation elicited by food-related images in the posterior inferior cerebellar hemispheres, most prominently at GM/white-matter boundaries in lobules IX and VIIIb (Table 2, top; Figure 2, middle panel). Post hoc analyses revealed a significant effect ($p < 0.001$) in 5.0 % of the voxels in lobule IX bilaterally, and 4.0 % of the voxels of lobule VIIIb. This effect overlapped with the area where we have shown covariation between duration of leptin replacement and GM structure [8]. At the voxel where the structural covariation was strongest ($-29, -57, -42$), duration of leptin replacement was also

correlated with the BOLD response to food-related cues in the present investigation ($t = 3.52$, $p < 0.002$). This observation supports the idea that reduction in GM structural signal when leptin is withheld may contribute to reduced local activation by food-related stimuli.

In contrast, brain activity elicited by food-related images was associated with higher BMI in a superior part of the posterior cerebellar hemispheres, in lobules VI and Crus I, with clear $L > R$ asymmetry, and in the precuneus of the posterior parietal lobe. Structural changes were not associated with either BMI or duration of leptin replacement in these areas [8]. Post hoc analyses indicated a significant effect ($p < 0.001$) in 10.1% of lobule VI bilaterally (17.6 % in the left cerebellar hemisphere) and 6.8% of Crus I bilaterally (11.0 % in the left cerebellar hemisphere). The corresponding association with duration of leptin replacement involved only 3.7 % of lobule VI bilaterally and 2.4 % of Crus I. Because cerebro-cerebellar connections are primarily contralateral, most language and motor tasks produce $R > L$ activation. However, $L > R$ activation in lobules VI and Crus I associated with emotional processing and executive tasks has been reported [11]. The precuneus has been linked with visual processing, anticipation, and imagery. We interpret the greater activity associated with BMI as representing an increased emotional response to food-related stimuli when leptin-deficiency increases hunger and body fat.

Cerebellar function has been implicated in the fine-tuning of sensorimotor processing, but an emerging reconceptualization implicates the posterior lobe of the cerebellum, where the current findings are localized, in higher-level cognitive and emotional tasks [11–13]. The cerebellum has also been shown to detect blood-borne nutritional signals directly, and to be activated during anticipation of food [40]. In six diet-regulated obese inpatients assessed before and after stabilization at a 10% reduced body weight, dieting was associated with increased fMRI-measured activation by visual food stimuli in the dorsal posterior lobe of the cerebellum, and leptin administration reversed the increase [41]. Since leptin also reversed the increased hunger associated with the weight-reduced state [41], the effect was consistent with the cerebellar activation associated with BMI that we observe and attribute to increased hunger when leptin was withheld [7]. While administration of leptin for five weeks to the weight-reduced inpatients, as compared to administration of placebo, decreased activation in dorsal posterior cerebellum, it also increased activation in nearby voxels [41]. The increases may have been mediated by leptin-associated increases in GM structure like those we have reported [8], since leptin was administered in doses designed to reverse the relative hypoleptinemia induced by weight reduction [41].

Although leptin also increased activation in extracerebellar structures of the inpatients, the participants were not leptin-deficient [41] although they may have been leptin-resistant. In addition, very different statistical thresholds were employed as compared to our study. In the final across-subjects analysis, a voxel threshold of $p < 0.05$ and a spatial extent threshold of 5 or more contiguous voxels were used [41]. We employed more conservative thresholds; voxel $p < 0.001$ with spatial extent $p < 0.05$ after multiple-comparisons correction for whole-brain search volume (>115 voxels). The z-scores for effects of leptin replacement corresponding to the T-scores in our Table 2 range from 3.83 to 4.57, as compared to scores ranging from 1.69 to 3.30 in Table 3 of the earlier study [41]. In our study, BOLD response was also directly related to the number of days of leptin replacement with peak Z-scores > 3 in most of the extracerebellar structures where leptin increased activation in the earlier study [41], but these effects did not survive multiple-comparison correction for whole-brain volume, and therefore cannot be interpreted as evidence without an a priori hypothesized effect location.

Although it has been argued that the high levels of OB-Rb leptin receptors in the human cerebellum may indicate a function unrelated to body weight homeostasis [42], our findings,

together with the other studies discussed above, suggest that both structural and functional effects of leptin on cerebellar plasticity may in fact be part of the mechanism by which leptin alters food consumption. They also underscore the importance of considering structural effects when interpreting functional brain response.

Extrahypothalamic effects of leptin are consistent with neurochemical actions, including activation of synaptic NR2A-containing *N*-methyl-D-aspartate (NMDA) receptors and the mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) in multiple brain areas where leptin regulates synaptic morphology [43–45]. The MAPK/ERK [46], phosphatidylinositol 3-kinase (PI3K) [47] and nuclear factor kappa-B transcription factor (NF- κ B) pathways [48] have been shown to contribute to pro-survival responses that make leptin a potent neurogenic factor in hippocampal [49, 50] and cortical neurons [51]. Through activation of a Janus kinase signal transducer and activator of transcription-3 (JAK/STAT3) / PI3K / Protein Kinase B (PKB) -signaling cascade, leptin protects hippocampal neurons from apoptosis induced by removal of trophic support and excitotoxic and oxidative insults [52]. In the cerebellum specifically, leptin promotes survival of Purkinje cells [10], and facilitates NMDA receptor-mediated calcium influx in granule cells [53], to activate the JAK2/STAT3 pathway and to reduce PKB activation [54]. At a systems level, effects of leptin on NMDA receptors could have extensive functional influence because of the wide distribution of these receptors in brain and their role in excitatory synaptic transmission.

Limitations of the current study include the rarity of congenital leptin deficiency, and the fact that common obesity is often accompanied by leptin resistance, as it was for our patient C. This patient required 13–17 times as much leptin daily as the others to achieve comparable weight loss, and has been less able than the others to maintain a stable BMI (see Table 1). The fact that this individual showed changes in brain response to food cues, despite relative hypothalamic leptin resistance, can be explained by the higher dosing and tissue-specificity of leptin resistance. In diet-induced, obese hyperleptinemic mice, the arcuate nucleus is the major site of leptin resistance, whereas other hypothalamic and extrahypothalamic sites remain leptin-responsive [55]. Since the first-level individual results for effects of leptin on brain function were similar in all three patients, patient C may be relatively more leptin-resistant at the level of the arcuate nucleus, but more leptin-responsive in extrahypothalamic regions such as the cerebellum.

Although the interaction of functional and structural effects of withholding leptin would be best quantified via a combined multimodal analysis, the small sample size precluded this type of analysis with currently available statistical tools [56, 57]. A strength of this study is that it evaluated the consequences of the complete absence of leptin, and of its replacement, both in the short- and long-term, in a human model.

In conclusion, this study demonstrates two opposite and reversible effects of leptin replacement on cerebellar response to food cues in leptin-deficient adults. Leptin supplementation decreases hunger and BMI [7]. In this study, the BMI decreases were associated with decreases in activity within lobules VI and Crus1 of the posterior cerebellar hemispheres after presentation of food-related stimuli (Table 2). We tentatively interpret this effect as due to decreased motivational salience of the stimuli when participants are not as hungry. At the same time, a longer as compared to a shorter duration of ongoing leptin supplementation was associated with more activation ventrally in cerebellar lobules IX, VIIIB and VI. This may be a consequence of the reversible increase in GM structure at these locations which we have shown to be produced by leptin in these patients [8].

More generally, these findings contribute to emerging evidence for plasticity of brain structure-function relationships over a few weeks [58] or even hours [59], and suggest that leptin may have therapeutic value in modulating plasticity-dependent brain functions. The results reported here also highlight the possibility of a hitherto underexplored but critical role for the cerebellum in the regulation of leptin-mediated cognitive processes related to food intake.

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Figure 1.

Statistical parametric maps quantify areas across subjects and conditions where the blood oxygen-level dependant (BOLD) signal elicited by high-calorie food pictures was higher than that elicited by pictures of brick walls. Colored areas represent voxel t-scores > 3.85 ($p < 0.001$ uncorrected). For illustrative purposes, results are superimposed on a representative but not study-specific T1 image in MNI space for transaxial slices 18 mm below the level of the anterior commissure (AC), at the level of AC, and 8 mm above the AC. CB cerebellum, MB midbrain, Amy amygdala, OCC occipital cortex, Hip hippocampus, Str striatum, INS insula, Thal thalamus, IFG inferior frontal gyrus.

Figure 2.

Statistical parametric maps quantify direct relationships of the duration of leptin replacement and Body Mass Index (BMI) to the blood oxygen-level dependent (BOLD) signal elicited by high-calorie food pictures, relative to brick walls. *Left column:* Areas where BOLD signal covaried with BMI. *Middle column:* Areas where BOLD signal covaried with duration of leptin replacement. Colored areas (neurological orientation) represent voxel t-scores > 3.01 ($p < 0.005$ uncorrected). Results are superimposed on a representative but not study-specific T1 image for sagittal slices 29 mm to the left of midline (top row), and coronal slices 50 mm (middle row) and 70 mm (bottom row) posterior to the anterior commissure. *Right column:* Green lines demarcate and white text labels the fissures and lobules of the cerebellum [60]. On the sagittal image, blue scale depicts areas where grey matter structure directly covaried

with duration of leptin replacement in these same patients during 2005, 2006 and 2007, reprinted with permission [8].

Table 1

Duration of leptin replacement and BMI at each fMRI session.

2006			2007		
Leptin Condition ^a at each fMRI session	BMI	Leptin Days	BMI	Leptin Days	Leptin Days
Patient A	+	24.0	>300	23.0	>300
Male	-	26.2	-32	25.9	-37
age 31 (2006)	Brief	26.1	14	26.5	23
Patient B	+	27.5	>300	27.3	>300
Female	-	29.3	-32	29.3	-37
age 40 (2006)	Brief	29.6	14	28.3	18
Patient C	+	31.6	>300	33.2	>300
Female	-	33.3	-32	36.7	-37
age 45 (2006)	Brief	33.4	14	36.9	23

^a condition + = over 10 months of daily leptin replacement; - = 32 to 37 days without leptin; Brief = 14–23 days after daily leptin was restored (see “Leptin Days” columns)

Table 2

Relationship of regional brain activation by food cues to days of contiguous leptin replacement and BMI.^a

Positive covariation of activity with days of leptin replacement					
Cluster Size		Peak Voxel			
p	k	t	location	structure	
.014	156	7.43	-22 -54 -44	Cerebellum (IX, VIIIB & VI)	
.011	166	5.89	-20 -70 -26	Cerebellum (VI)	
.043	121	5.37	18 -52 -50	Cerebellum (IX & VIIIB)	

Positive covariation of activity with BMI					
Cluster Size		Peak Voxel			
p	k	t	location	structure	
.0005	732	7.43	-20 -54 -20	Cerebellum (VI & Crus I)	
.048	117	7.18	24 -78 38	Precuneus (B19)	

^aClusters of contiguous voxels from a whole-brain analysis with voxel $p < 0.001$ (uncorrected)

p = whole-brain corrected spatial extent (cluster size) probability, k = number of 2×2×2 mm voxels, t = Student's t-statistic