

Reward Processing During Monetary Incentive Delay Task After Leptin Substitution in Lipodystrophy – an fMRI Case Series

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Abstract

Context: Behaviorally, the most pronounced effects of leptin substitution in leptin deficiency are the hunger-decreasing and postprandial satiety-prolonging effects of the adipokine. Previously, with functional magnetic resonance imaging (fMRI), we and others showed that eating behavior-controlling effects are at least in part conveyed by the reward system. However, to date, it is unclear if leptin only modulates eating behavior specific brain reward action or if it also alters the reward function of the brain unrelated to eating behavior.

Objective: We investigated with functional MRI the effects of metreleptin on the reward system in a reward task unrelated to eating behavior, the monetary incentive delay task.

Design: Measurements in 4 patients with the very rare disease of lipodystrophy (LD), resulting in leptin deficiency, and 3 untreated healthy control persons were performed at 4 different time points: before start and over 12 weeks of metreleptin treatment. Inside the MRI scanner, participants performed the monetary incentive delay task and brain activity during the reward receipt phase of the trial was analyzed.

Results: We found a reward-related brain activity decrease in our 4 patients with LD over the 12 weeks of metreleptin treatment in the subgenual region, a brain area associated with the reward network, which was not observed in our 3 untreated healthy control persons.

Conclusions: These results suggest that leptin replacement in LD induces changes of brain activity during reward reception processing completely unrelated to eating behavior or food stimuli. This could suggest eating behavior-unrelated functions of leptin in the human reward system.

Trial registration: The trial is registered as trial No. 147/10-ek at the ethics committee of the University of Leipzig and at the State Directorate of Saxony (Landesdirektion Sachsen).

Key Words: leptin, lipodystrophy, reward system, monetary incentive delay task, functional MRI

Abbreviations: BMI, body mass index; HbA1c, hemoglobin A1c; LD, lipodystrophy; MIDT, monetary incentive delay task; MRI, magnetic resonance imaging; MNI, Montreal Neurological Institute; SPM, statistical parametric mapping.

The adipocyte-derived hormone leptin is an important and strong regulator of eating behavior [1]. As for other bodily hormones regulating food intake, such as glucagon-like peptide-1 [2] or ghrelin [3], a main site for its action on eating behavior is the hypothalamus. Here, hormones from the periphery can enter the brain through the leaky blood–brain barrier and exert their effects on the central nervous system [4]. The hypothalamus is considered the center of control for the homeostatic component of eating regulation in humans, which is the sum of the stimuli for food intake to satisfy the body's demands for energy [5]. This homeostatic component

is often contrasted with the hedonic component of eating behavior, which is the pleasure of eating in the absence of fuel need.

In animal models, distinct neuron populations could be identified that mediate homeostatic signaling and are directly modified by leptin: in the arcuate nucleus of the hypothalamus, leptin inhibits orexigenic neuropeptide Y and agouti-related peptide neurons [6] and activates anorexigenic cocaine- and amphetamine-regulated transcript and proopiomelanocortin neurons [7]. Previously, we [8] and others [9] demonstrated leptin action in the human hypothalamus in

regulating eating behavior using functional magnetic resonance imaging (MRI). In obesity, because of the increased fat mass resulting in high leptin blood concentrations [10], leptin resistance occurs. Leptin resistance leads to decreased leptin signaling in the hypothalamus and thus to reduced anorexigenic effects of leptin on eating behavior [11].

With leptin deficiency, which occurs in the rare congenital or acquired lipodystrophies (LD) [12] or the extremely rare disease congenital leptin deficiency [13], patients have increased feelings of hunger and decreased duration of satiety after eating. Leptin substitution in these patients leads to a normalization of eating behavior: perceived hunger, importance of eating, eating frequencies, and liking ratings of food pictures significantly decrease [8, 14].

However, leptin does not only seem to influence eating behavior by regulating the homeostatic control of eating behavior through the hypothalamus. It also influences the control of food intake through the reward system [15, 16]. With functional neuroimaging, the neural correlates in the reward network and associated brain regions in leptin-deficient patients undergoing leptin replacement have been identified using tasks that activate the networks regulating eating behavior [16–19]. However, it is unclear if the effects of leptin on the central nervous system are specific for the regulation of food intake or if leptin also affects reward processes when they are unrelated to eating behavior. In our study, we tested if metreleptin substitution in female patients with congenital partial LD led to changes in brain activation when participants were performing the monetary incentive delay task (MIDT), a classic task leading to robust activation of the reward system [20]. To control for nonspecific order effects, we also performed the same measurements in an untreated healthy control group.

We found a reward-related brain activity decrease in our 4 LD patients over the 12 weeks of metreleptin treatment, which did not occur in our 3 untreated healthy control persons, in the subgenual region, a brain area associated with the reward network. This could suggest leptin induced changes of brain activity during reward reception processing, which are completely unrelated to eating behavior or food stimuli.

Research Design and Methods

LD Patients

Four patients with LD (all cis-female by self-report) eligible for metreleptin treatment at the University Hospital Leipzig participated in the functional MRI study. Baseline characteristics and laboratory data of included patients are summarized in Table 1, together with data of healthy control persons. Inclusion criteria for leptin substitution were established LD, age ≥ 5 years at baseline, insufficiently controlled diabetes mellitus, and/or hypertriglyceridemia despite adequate antihyperglycemic and lipid-lowering medication, respectively. Exclusion criteria included pregnancy or lactation, severe renal insufficiency, active malignant disease, primary hematologic abnormalities, infectious liver disease, HIV infection, and hypersensitivity to *Escherichia coli*-derived proteins. All 4 patients had familial partial LD with mutations in the lamin A/C gene and were metreleptin treatment naïve. They all consented to participating in the MRI study. Healthy control persons were selected by matching sex (all female), age, and body mass index (BMI) range of patients with LD.

Medication

The leptin analogue metreleptin was used for treatment. Metreleptin was provided by Amylin (San Diego, CA)/Bristol-Myers-Squibb (Munich, Germany)/AstraZeneca (London, UK)/Aegerion Pharmaceuticals (Cambridge, MA), respectively, and applied subcutaneously. Metreleptin was administered once daily at 5 mg independent of body weight, according to manufacturer's instructions.

Experimental Design

Experiments were performed between July 2017 and March 2020. Behavioral tests and MRI scans in patients with LD were performed at 4 timepoints (ie, 1 day before start of metreleptin supplementation [T0], and after 1 [T1], 4 [T2], and 12 weeks [T3]) of metreleptin treatment. In healthy control persons, 4 measurements were conducted at the same intervals (Supplementary Fig. S1A [24]). Procedures at each measurement day for patients with LD and healthy control persons were identical: participants arrived at the institution at about 1330 hours. Questionnaires and behavioral tests were performed, as detailed later. Then, a standard meal consisting of 20% of the daily energy requirements calculated for each participant was consumed. Because leptin physiologically mediates satiety, differences between leptin deficiency and imitated physiological leptin concentrations from metreleptin treatment were expected to be most pronounced in the sated state and, therefore, the mentioned calorie amount was chosen to create a state of moderate satiety. We did not choose a higher percentage of daily energy requirements to avoid both a ceiling effect in extreme fullness and postprandial tiredness during the following functional MRI scan. At around 1530 hours, the MRI scan was performed (Supplementary Fig. S1B [24]). In patients with LD, a fasting blood sample was taken the next morning at 8 AM.

Monetary Incentive Delay Task

The MIDT was performed inside the MRI scanner. The experiment consisted of 90 trials. At the beginning of each trial, a cue was presented indicating the possible reward for this trial. The 3 different trial categories of high reward (high; 50 Euro cents), low reward (low; 20 Euro cents), and no reward (neutral; 0 Euro cents) were used, with 30 trials each. The order of the 90 trials was randomized. After presentation of the cue (250 ms), participants would press a button as fast as they could when the target (a black square) appeared on the screen. The anticipation time between the cue and the target was randomized between 2250 and 2750 ms. Then, 500 ms after pressing the response button, the information, if they had pressed the button fast enough to receive the reward or not, and the amount gained in this trial and the total sum was displayed (receipt phase, 1667 ms). After an intertrial interval of 4000 to 6000 ms (randomized), the next trial was started. The reaction time needed to gain the reward was automatically adapted during the task so that about 2/3 of the trials were won and 1/3 lost, of which participants were not aware. The initial threshold was 2750 ms and the maximum adaptation between 2 trials was ± 500 ms. In the middle of the experiment, there was a pause for rest for 60 seconds to give the participants time to relax. The whole reward experiment lasted 17 minutes and 20 seconds. Immediately after participants left the MRI scanner, the reward was paid, which was about 14 Euros.

Table 1. Baseline characteristics and laboratory data of the study population

	Age (y)	BMI (kg/m ²)	Education (school years)	Apprenticeship/university	Phenotype of LD	Mutation	Leptin (µg/L)
Patient 1	52	28.3	10	1-y apprenticeship	Partial	LMNA	3.4
Patient 2	45	25.0	9	3-y apprenticeship	Partial	LMNA	2.3
Patient 3	26	21.0	12	3-y apprenticeship	Partial	LMNA	1.3
Patient 4	26	33.9	10	3-y apprenticeship	Partial	LMNA	3.9
Control 1	57	35.7	10	3-y apprenticeship	None	—	58.4 ^a
Control 2	43	29.8	10	3-y apprenticeship	None	—	43.9 ^a
Control 3	28	24.0	12	2nd year university at T0	None	—	4.5 ^a

All participants were female. Education: in Germany, school education can be done in 9 years (“*Hauptschule*,” lowest level), 10 years (“*Realschule*”), or 12/13 years (“*Gymnasium*,” usually needed to be admitted to university). Leptin serum concentrations are sex, age, and BMI dependent and correlate with the percentage of body fat [10, 21, 22]. In patients with lipodystrophy, leptin concentrations are usually lower than in healthy persons [23].

Abbreviations: BMI, body mass index; LD, lipodystrophy; LMNA, lamin A/C; T0, baseline measurement, T3, measurement after 12 weeks.

^aLeptin values in healthy control persons were assessed at T3. Between T0 and T3, in healthy control persons, there was no intervention and body weight changes during that interval were <2 kg.

After reaction time assessment, a plausibility check was performed, and all trials removed that had reaction times <100 ms and >500 ms. Then, outliers deviating more than 2.5 SD from the mean were excluded. A graphical check showed normal distribution of the data.

MRI Brain Data Acquisition

Functional brain data were obtained using functional MRI with a whole-body 3-T MAGNETOM Skyra scanner (Siemens Healthineers, Erlangen, Germany) with a 32-channel head coil and a gradient-echo echo-planar imaging sequence (multiband factor 3). The following parameters were used: 530 functional brain volumes, acquisition matrix = 82 × 82, and slice thickness = 2.5 mm (0.25-mm gap), resulting in a nominal voxel size of 2.488 × 2.488 × 2.75 mm³. Further imaging parameters were: 60 axial slices, repetition time = 2000 ms, echo time = 22 ms, flip angle = 80°, and bandwidth = 1795 Hz/pixel. During scanning, participants had to perform the MIDT described previously. Using a total of 530 functional volumes, the length of the functional experiment was 17 minutes and 20 seconds.

MRI Data Preprocessing

All functional MRI data sets were analyzed using the CONN toolbox rev. 20b [25, 26] and statistical parametric mapping (SPM) 12 rev. 7771 [27] (Wellcome Centre for Human Neuroimaging, University College London, UK) with Matlab 9.12 rev. R2022a (The MathWorks Inc., Natick, MA). Preprocessing was performed using the default SPM pipeline within the CONN toolbox [25], including realignment for motion correction, unwarping of echo-planar imaging to correct for distortions, slice-time correction, and normalization to the Montreal Neurological Institute (MNI) space based on the unified segmentation approach [28]. Normalization was performed with the default settings for resampling and interpolation using a destination resolution of 2 × 2 × 2 mm³. Thereafter, spatial filtering was applied using a Gaussian kernel with 10-mm full width at half maximum. Image preprocessing also included denoising within the CONN toolbox. To correct for nuisance signal fluctuations, a regression analysis was computed using the signal from regions of cerebrospinal fluid (16 regressors) and the

translational and rotational parameters from head movements obtained by SPM 12 (24 regressors). Preprocessing was finalized using linear detrending and high-pass filtering using a cutoff frequency of 0.012 Hz to achieve a baseline correction.

MRI Data Statistical Analysis

For each participant and each measurement, each data set was further processed by least-squares parameter estimation using the general linear model with serially correlated observations (first-level analysis) [29, 30]. Two different analyses were performed: “anticipation” and “reward.”

The “anticipation” analysis was aimed at checking the validity of the experimental setup, the data, and the analysis procedure because of a clear hypothesis expecting brain activity increase with an anticipation of “high” reward in the nucleus accumbens. Here, we investigated brain activity differences right after showing the initial cue that indicated the possible reward for the trial. Thus, the design matrix was generated using the onsets of the initial cue of all 3 experimental conditions: high, low, and neutral. Using the standard model in SPM12, the stimulus function (generated from the onsets) was convolved with a canonical hemodynamic response function [31], including its first derivative resulting in 2 columns for each condition. For each patient, the 4 different sessions T0 to T3 were merged into a single design using a fixed-effects model (ie, the sessions were used as 4 diagonal blocks in an extended design matrix). Parameter maps (beta images) were estimated and contrast images were generated by subtracting the conditions high-neutral. Subsequently, for each patient, a first-level statistical analysis was performed to assess significant brain activity differences using an extremely conservative approach using a voxel threshold of $P < .01$ with family-wise error correction at the voxel level. Finally, thresholded individual statistical maps were merged into a group result including the overlap of all patients, which was further overlaid with a map showing the functional location of the nucleus accumbens obtained by an automated meta-analysis using 194 studies in neurosynth.org [32]. Using the key word “nucleus accumbens” with an association test, Z scores were obtained from a 2-way ANOVA testing for the presence of a non-zero association between term use and voxel activation. Note that this meta-analysis was also corrected for multiple

comparisons using a false discovery rate of 0.01 and finally processed with a voxel threshold of $Z > 10$.

The second analysis “reward” was aimed at investigating the temporal changes of reward related brain activity with metreleptin administration. First-level analysis was performed using least-squares parameter estimation using the general linear model for each participant and each session separately. In contrast to the anticipation analysis described previously, the reward analysis comprised 5 experimental conditions depending on the initial cue for the potential reward and the actual reward presented in the receipt phase in the end of the trial: HH (reward with initial high reward cue); LL (reward with initial low reward cue); HN (no reward with initial high reward cue); LN (no reward with initial low reward cue), and NN (no reward with initial neutral cue). The design matrix was then generated using the onsets of the receipt phase with all 5 experimental conditions HH, LL, HN, LN, and NN. Similar to the first analysis, the stimulus function (generated from the onsets) was convolved with a canonical hemodynamic response function including its first derivative resulting in 2 columns for each condition. Parameter maps (beta images) were estimated, and reward contrast images were generated by “reward – no reward” by subtracting the conditions (HH, LL) – (HN, LN). Finally, a contrast image was obtained for each participant and each session (ie, 28 contrast images were processed in subsequent second-level group analyses). Note that all group analyses were performed using uncorrected voxel thresholds and no *P* values were reported because of the limitation of the very low number of participants.

The first “reward” group analysis was performed using a general linear model with the flexible factorial design including the contrast images of all patients introducing a factor “time” (4 longitudinal measurements). The *F*-contrast matrix comprised all measurements (identity matrix). The resulting *F*-map was further processed with an uncorrected voxel threshold of $F > 12.56$ and a cluster size threshold of $k > 200$.

The second “reward” group analysis was aimed at investigating a decay of reward-related brain activity over time specifically to patients with LD. Therefore, a group analysis was performed using a general linear model with the flexible factorial design including the factors “group” (between participants; patients, controls) and “time” (within participants, 4 measurements). Because our primary hypothesis was a decay of reward-related brain activity over time ($C = [1\ 0\ 0\ -1]$) specifically to patients with LD, we first tested for a potential interaction between both factors group and time using the *T*-contrast $[C, -C]$. Thereafter, in a post hoc fashion, we tested for a potential decrease of reward-related brain activity over time in patients and controls separately. Resulting parametric maps were processed using an uncorrected voxel-wise threshold of $T > 3.73$ with a minimum cluster size of $k > 50$ and $k > 200$ for the interaction and post hoc analyses, respectively.

Results

Anthropometric and Metabolic Parameters

Baseline characteristics at T0 of all patients and untreated healthy control participants are summarized in Table 1. Patients with LD were 26 to 52 years old and the BMI range was 21.0 to 33.9 kg/m². Baseline leptin serum concentrations ranged from 1.3 to 3.9 μg/L, hemoglobin A1c (HbA1c) from 6.1% to 9.2% (43-77 mmol/mol) and serum triglyceride

concentrations from 1.5 to 41.5 mmol/L. Healthy control participants were aged 28 to 57 years; BMI range was 24.0 to 35.7 kg/m². Laboratory values in healthy control participants were only measured at T3. Leptin serum concentrations in control participants ranged from 4.5 to 58.4 μg/L, HbA1c from 5.0% to 5.4% (31-36 mmol/mol), and serum triglyceride concentrations from 0.5 to 4.6 mmol/L. As in our previous, larger study [8], in patients with LD, metreleptin treatment led to a mild decrease in BMI and improvements in HbA1c and fasting triglycerides on an individual level (data not shown). Between T0 and T3, in healthy control persons, there was no intervention and body weight changes during that interval were <2 kg.

Reaction Times in Monetary Incentive Delay Task

Mean reaction times in the high reward (50 Euro cents) condition in patients with LD were from T0 to T3 2532 ms, 2457 ms, 2458 ms, and 2457 ms, in the low reward (20 Euro cents) condition 2605 ms, 2581 ms, 2529 ms, and 2469 ms, and in the no reward (neutral, 0 Euro cents) condition 2897 ms, 2802 ms, 2532 ms, and 2673 ms. In untreated healthy control persons, mean reaction times in the high reward condition were 233.9 ms, 219.4 ms, 224.8 ms, and 228.1 ms, in the low reward condition 233.7 ms, 224.4 ms, 229.1 ms, and 226.7 ms, and in the no reward condition 248.9 ms, 228.8 ms, 237.4 ms, and 232.4 ms. Differences in reaction times high reward – no reward (“neutral”) for all 4 LD patients and all 3 control persons are shown in Fig. 1.

Functional MRI Data

The anticipation analysis showed a brain activity difference with the high-neutral contrast in both the left and right ventral striatum in all 4 patients with LD (see “Patients” in Fig. 2 showing the overlap of the high-neutral contrast of all 4 patients with LD). According to our meta-analysis using neurosynth.org [32], our finding was located in the left and right nucleus accumbens (see “NAcc” in Fig. 2), which demonstrates the expected brain activity difference with the experimental setup using our reward-related paradigm.

The reward analysis at full-brain level (ie, for all voxels of the brain) including all patients revealed a change of reward-related brain activity during metreleptin administration in the subgenual area (Brodmann area 25) [33] bilaterally. This analysis did not reveal any other statistically significant changes of brain activity during the 12 weeks of therapy (Fig. 3). As the design comprised the reward difference (reward – no reward) in the first-level analysis, and the time factor in the second-level design, the result shows an interaction between both factors reward and time.

A second reward analysis was aimed at investigating potential group differences using all participants with an extended model including the factors time and group. To further take our initial hypothesis into account, we used a *T*-contrast C to model brain activity decay over time. Investigating the patients, in line with our previous results, we obtained a brain activity decrease over time in the subgenual area bilaterally, but nowhere else in the brain (Fig. 4A). For the untreated healthy control persons, we did not find any results across the whole brain (Fig. 4B, Supplementary Fig. S2 [24]). Using the extended *t*-contrast $[C, -C]$, we found an interaction between both factors time and group, showing a difference between patients and controls with respect to the reward-related

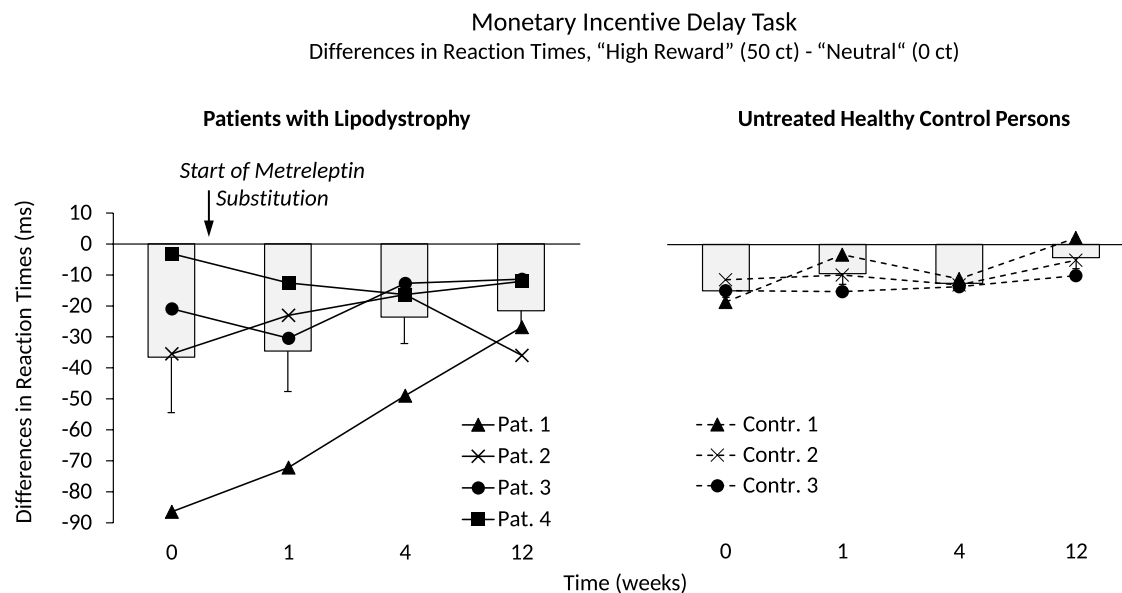


Figure 1. Differences in reaction times in monetary incentive delay task between the categories high reward (50 Euro cents)—no reward ("neutral", 0 Euro cents). Left: patients with lipodystrophy; right, untreated healthy control participants. Bars are mean values with standard error of the mean for all 4 patients with LD (left) and all 3 control persons (right), respectively. Contr., control person, Pat., patient.

brain activity change over time. Here, we obtained a cluster in the subgenual area bilaterally (Fig. 4C). The interaction analysis revealed a second cluster in the cerebellum (not shown) that was not obtained when looking at patients and controls separately.

Discussion

In our study, we investigated the effects of metreleptin substitution in leptin-deficient patients with LD on brain activity during the reward receipt of the classical reward task MIDT.

With functional MRI, in eating behavior-related tasks, brain activity changes in the reward network and its associated regions were demonstrated. In a study with patients with congenital leptin deficiency, brain activations when rating food pictures in reward areas (specifically, in nucleus accumbens and caudate nucleus), whereas fasting correlated with liking ratings of food pictures in both the metreleptin treated and untreated (ie, leptin-deficient) state. However, in the fed state, this correlation could not be found during metreleptin treatment but remained statistically significant in the metreleptin-untreated state. This suggests that leptin is necessary to suppress the incentive motivational value to food stimuli in the fed state [16].

Unlike previous functional MRI studies with metreleptin substitution, we used a task that is unrelated to eating behavior. In patients with LD, we found a reward-related brain activity decrease in the subgenual area (Brodmann area 25) during the first 12 weeks of metreleptin substitution of female patients with LD, during the receipt phase of the task. The subgenual area is located caudally of the genu of the corpus callosum and part of the cytoarchitecturally defined cingulate region of the brain [34]. The area is considered part of the ventral limbic region [35] and has, among others, projections to the dorsolateral prefrontal cortex.

In our group of 4 patients with LD, in contrast to changes in brain activity, we did not see robust changes in reaction times in MIDT over the time course of the study. This was also the

case in other functional MRI studies using the MIDT as neuro-behavioral task. In a study investigating the effects of hunger/satiety on MIDT outcomes including 36 trials into the analysis (10 female and 8 male participants; BMI 18-29 kg/m²; age 18-50 years, each participant measured twice: fasted and post-prandial), no effects of ingestive state on reaction times and no interaction with reward magnitude was found, but functional MRI data during the anticipation phase (from presentation of the possible reward until the participants' response [ie, different than in our study in which we assessed brain function in the receipt phase]), showed a greater signal increase with higher rewards in the fasting condition. Authors interpreted their findings as an increased incentive-related brain activity for nonfood rewards during fasting [36].

In another study including 54 trials of healthy volunteers of a higher age resulted in slower reaction times in the high-reward condition (1 US dollar), but not in the low-reward condition (1 US cent), and age was associated with diminished differences in reaction times to acquire a large vs small reward. Functional MRI data during the receipt phase of the MIDT showed that activations in the thalamus to high vs small reward decreased with age [37].

The underlying mechanisms of how metreleptin decreases reward-related brain activity were not investigated in this study. To our knowledge, in neurons of the subgenual region, the leptin receptor has not yet been identified; thus, a neuronal transmission of the information starting from the leptin receptor-holding neurons of the hypothalamus or the mesolimbic dopamine system is the most plausible mechanism of leptin signal transmission to the subgenual region of the brain.

Interestingly, in a recent functional MRI study investigating the beneficial effects of cognitive behavioral therapy in patients with major depressive disorder, a reduction in anhedonia severity posttreatment was associated with enhanced activations in subgenual anterior cingulate cortex and nucleus accumbens during the receipt phase of the MIDT [38]. The noneating reward-related decrease in subgenual anterior cingulate cortex activity we saw in our patients were also

Anticipation: High > Neutral

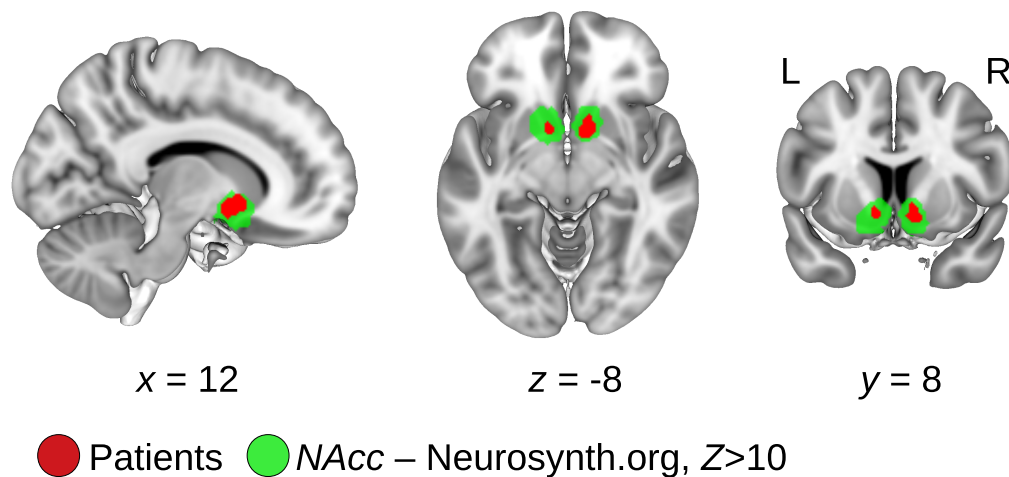


Figure 2. Orthogonal brain slices showing a brain activity difference in the anticipation phase of the monetary incentive delay task contrasting the conditions high reward (high, 50 Euro cents) vs no reward (neutral, 0 Euro cents) for all lipodystrophy patients (see regions denoted by “Patients”). Regions denoted by “NAcc” show the result of a meta-analysis using 194 studies in neurosynth.org [32] with the keyword “nucleus accumbens.” Z scores were obtained from a 2-way ANOVA testing for the presence of a non-zero association between term use and voxel activation. L, left; MNI, Montreal Neurological Institute; NAcc, nucleus accumbens; R, right; x, y, z, MNI coordinates in millimeters.

Reward * Time

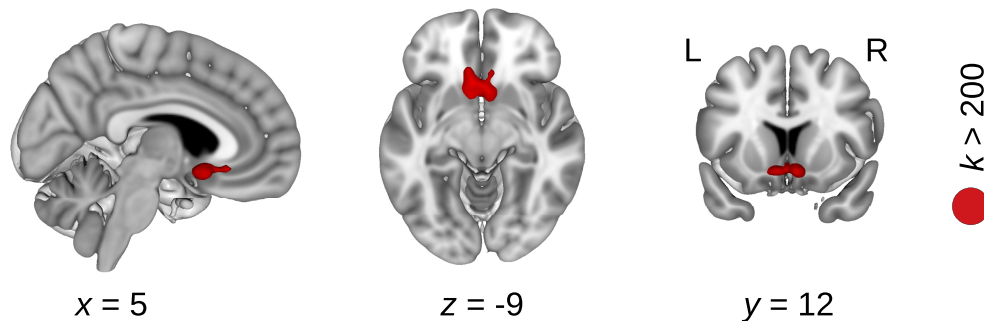


Figure 3. Orthogonal brain sections showing brain activity change during metreleptin treatment in patients with lipodystrophy. Brain activity differences were assessed during the receipt phase of the monetary incentive delay task with the interaction between the factors “reward” (reward, ie, 50 Euro cents + 20 Euro cents conditions vs no-reward [ie, 0 Euro cents]) and “time” using an uncorrected voxel threshold of $F > 12.56$ and a minimum cluster size of $k > 200$. L, left; MNI, Montreal Neurological Institute; R, right; x, y, z, MNI coordinates in millimeters.

associated with mood improvements. We thus cannot exclude that MIDT activation changes do not reflect a direct effect of metreleptin on reward processing, but rather a change that occurs indirectly due to effects of metreleptin substitution on the mood of treated patients with LD.

To relate our current brain activity findings while performing the classical reward task MIDT with our previous, task-free (resting state), functional MRI findings also obtained in a cohort of patients with LD under metreleptin treatment [8], we plotted the results of these 2 independent studies in an additional figure (Supplementary Fig. S3 [24]). Results show an overlap of the results of the two experiments. However, conclusions about exact localizations of involved neuron populations are not possible with functional MRI because this overlap only describes a colocalization in a mesoscopic resolution. Nevertheless, changes in resting state connectivity observed in previous work [8] may be related

to changes in brain activity observed during performance of the MIDT found in the current study. Still, considering the available human functional MRI data, this is speculative.

We also need to discuss a few limitations of our study. First, during the recruitment time of the study, because of the rarity of the disease of LD, we could only include 4 patients with LD into the study, together with the 3 control persons, resulting in a rather small sample for a functional MRI study. Because LD is very rare, all published functional MRI studies with metreleptin treated initially leptin-deficient patients (because of LD or congenital leptin deficiency) are small ranging from $n = 1$ to 10 [8, 13, 16, 18, 19]. Nevertheless, these studies were all able to detect major effects of metreleptin therapy, including ours. This is reassuring for the strength of the effects of leptin with its crucial functions on metabolism and cognition. Other examples of hormones with comparably strong effects on cognition and metabolism are insulin and glucagon. For example,

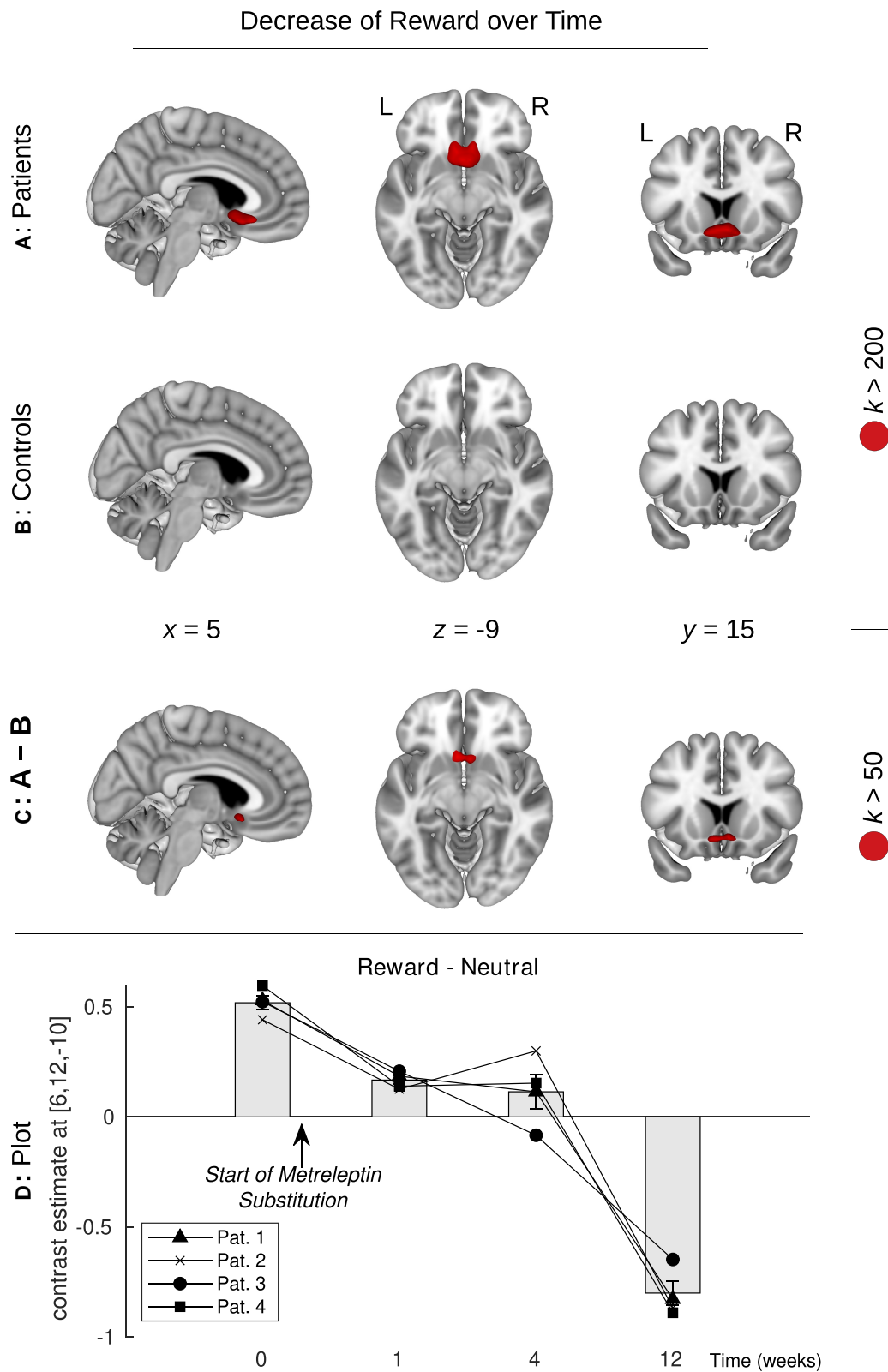


Figure 4. Orthogonal brain sections showing brain activity in lipodystrophy patients using a flexible factorial model with the factors “group” (patients; controls) and “time” (4 measurements). Brain activity differences were assessed during the receipt phase of the monetary incentive delay task, with the reward contrast using an uncorrected voxel threshold of $T > 3.73$ and a minimum cluster size of $k > 200$. **(A)** A reward-related brain activity decrease over time was obtained in patients in the subgenual area (Brodmann area 25) [33] bilaterally, but nowhere else in the entire brain. **(B)** No results were found with untreated control persons. **(C)** An interaction between the factors group and time was obtained in the same region shown in (A); however, the result was only obtained with reducing the minimum cluster size to $k > 50$. **(D)** Contrast estimates at the local maximum with the MNI coordinates [6 12 -10] for 4 individual patients with lipodystrophy (lines) and patient group averages \pm standard error (bars) for all 4 measured timepoints. Note that the contrast estimates represent a region around the local maximum according to spatial filtering applied in the preprocessing using a Gaussian kernel of 10-mm full width at half maximum. L, left; MNI, Montreal Neurological Institute; R, right; x, y, z, MNI coordinates in millimeters.

when treating patients with type 1 diabetes and absolute insulin deficiency, many effects of insulin substitution are already unmistakably seen even in single treated patients. Of note, decreases in brain activity observed in our study were found in every of our measured patients with very similar courses over time (Fig. 4D). Furthermore, because 4 measurements were performed in each patient, the total number of measurements included into our model was $n = 16$, taken together with the data of health controls the total number of measurements (Fig. 4) included into the interaction model was $n = 28$.

Second, our study was not a randomized study with a placebo-treated control group of patients with LD, but our control group were metreleptin-untreated healthy persons, without diabetes, hypertriglyceridemia, or respective medication. Because the health benefits of metreleptin treatment in LD are well documented and metreleptin therapy is the established therapy in patients with LD fulfilling the criteria for treatment initiation, a placebo-treated LD cohort would have been unethical. Because our study only investigated patients with LD and its comorbidities such as poorly controlled diabetes and hypertriglyceridemia, treated with respective medication, the obtained observations are specific for this patient group.

To conclude, in our study with 4 patients with LD over the 12 weeks of metreleptin substitution, we found a reward-related brain activity decrease in the subgenual anterior cingulate cortex, which did not occur in untreated healthy control persons. The subgenual region is a brain area strongly connected with the reward network, and we interpreted findings as direct alterations of the reward network through leptin. Thus, our findings suggest leptin induced changes of brain activity during reward reception processing, which were completely unrelated to eating behavior or food stimuli. Further studies should be conducted to assess which behavioral effects leptin deficiency as in our patients and subsequent leptin substitution has on reward function of the brain.

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Author Contributions

H.S. undertook conceptualization, data analysis, conduction of the study, project administration, and writing, reviewing, and editing of the manuscript. L.J. provided data analysis and writing, reviewing, and editing of the manuscript. A.V. undertook funding acquisition, resources, and reviewing of the manuscript. K. Miehle provided reviewing and editing of the manuscript. M.F. undertook conceptualization, funding acquisition, and reviewing of the manuscript. M.S. provided funding acquisition, resources, and reviewing of the manuscript. K. Mueller undertook conceptualization, data analysis, and writing, reviewing, and editing of the manuscript.

Data Availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

Statement of Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The Ethics Committee of the University of Leipzig approved this research project (approval no. 147/10-ek), according to the national research ethics regulations. All participants gave their written consent for all study procedures.

References

- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*. 1994;372(6505):425-432.
- Schlögl H, Kabisch S, Horstmann A, et al. Exenatide-induced reduction in energy intake is associated with increase in hypothalamic connectivity. *Diabetes Care*. 2013;36(7):1933-1940.
- de Guia RM, Hassing AS, Skov LJ, et al. Fasting- and ghrelin-induced food intake is regulated by NAMPT in the hypothalamus. *Acta Physiol (Oxf)*. 2020;228(4):e13437.
- van Swieten MMH, Pandit R, Adan RAH, van der Plasse G. The neuroanatomical function of leptin in the hypothalamus. *J Chem Neuroanat*. 2014;61-62:207-220.
- Yu Y-H, Vasselli JR, Zhang Y, Mechanick JI, Korner J, Peterli R. Metabolic vs. hedonic obesity: a conceptual distinction and its clinical implications. *Obes Rev*. 2015;16(3):234-247.
- van den Top M, Lee K, Whyment AD, Blanks AM, Spanswick D. Orexin-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. *Nat Neurosci*. 2004;7(5):493-494.
- Satoh N, Ogawa Y, Katsuura G, et al. Satiety effect and sympathetic activation of leptin are mediated by hypothalamic melanocortin system. *Neurosci Lett*. 1998;249(2-3):107-110.
- Schlögl H, Müller K, Horstmann A, et al. Leptin substitution in patients with lipodystrophy: neural correlates for long-term success in the normalization of eating behavior. *Diabetes*. 2016;65(8):2179-2186.
- Rosenbaum M, Sy M, Pavlovich K, Leibel RL, Hirsch J. Leptin reverses weight loss-induced changes in regional neural activity responses to visual food stimuli. *J Clin Invest*. 2008;118(7):2583-2591.
- Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med*. 1996;334(5):292-295.
- Wauman J, Tavernier J. Leptin receptor signaling: pathways to leptin resistance. *Front Biosci (Landmark Ed)*. 2011;16(7):2771-2793.
- Quinn K, Chauhan S, Purcell SM. *StatPearls: Lipodystrophies*. 2022.
- Farooqi IS, O'Rahilly S. 20 Years of leptin: human disorders of leptin action. *J Endocrinol*. 2014;223(1):T63-T70.
- Püschel J, Miehle K, Müller K, et al. Beneficial effects of leptin substitution on impaired eating behavior in lipodystrophy are sustained beyond 150 weeks of treatment. *Cytokine*. 2019;113:400-404.
- Sternson SM, Eisel A-K. Three pillars for the neural control of appetite. *Annu Rev Physiol*. 2017;79:401-423.
- Farooqi IS, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher PC. Leptin regulates striatal regions and human eating behavior. *Science (New York, N.Y.)*. 2007;317(5843):1355.

17. Baicy K, London ED, Monterosso J, *et al.* Leptin replacement alters brain response to food cues in genetically leptin-deficient adults. *Proc Natl Acad Sci USA.* 2007;104(46):18276-18279.
18. Frank S, Heni M, Moss A, *et al.* Leptin therapy in a congenital leptin-deficient patient leads to acute and long-term changes in homeostatic, reward, and food-related brain areas. *J Clin Endocrinol Metab.* 2011;96(8):E1283-E1287.
19. Frank S, Heni M, Moss A, *et al.* Long-term stabilization effects of leptin on brain functions in a leptin-deficient patient. *PLoS One.* 2013;8(6):e65893.
20. Knutson B, Westdorp A, Kaiser E, Hommer D. fMRI visualization of brain activity during a monetary incentive delay task. *NeuroImage.* 2000;12(1):20-27.
21. Zhou X, Chai Y, Chen K, Yang Y, Liu Z. A meta-analysis of reference values of leptin concentration in healthy postmenopausal women. *PLoS One.* 2013;8(8):e72734.
22. Isidori AM, Strollo F, Morè M, *et al.* Leptin and aging: correlation with endocrine changes in male and female healthy adult populations of different body weights. *J Clin Endocrinol Metab.* 2000;85(5):1954-1962.
23. Haque WA, Shimomura I, Matsuzawa Y, Garg A. Serum adiponectin and leptin levels in patients with lipodystrophies. *J Clin Endocrinol Metab.* 2002;87(5):2395.
24. Schlögl H, Janssen L, Fasshauer M, *et al.* Supplementary material for reward processing during monetary incentive delay task after leptin substitution in lipodystrophy—an fMRI case series. repository accession number.
25. Whitfield-Gabrieli S, Nieto-Castanon A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect.* 2012;2(3):125-141.
26. Nieto-Castanon A. *Handbook of Functional Connectivity Magnetic Resonance Imaging Methods in CONN.* Hilbert Press; 2020.
27. Friston KJ, Ashburner JT, Kiebel SJ, Nichols TE, Penny WD. *Statistical Parametric Mapping: the Analysis of Functional Brain Images.* Academic Press, Elsevier, 2006.
28. Ashburner J, Friston KJ. Unified segmentation. *NeuroImage.* 2005;26(3):839-851.
29. Friston KJ, Glaser DE, Henson RNA, Kiebel S, Phillips C, Ashburner J. Classical and Bayesian inference in neuroimaging: applications. *NeuroImage.* 2002;16(2):484-512.
30. Friston KJ, Penny W, Phillips C, Kiebel S, Hinton G, Ashburner J. Classical and Bayesian inference in neuroimaging: theory. *NeuroImage.* 2002;16(2):465-483.
31. Friston KJ, Fletcher P, Josephs O, Holmes A, Rugg MD, Turner R. Event-related fMRI: characterizing differential responses. *NeuroImage.* 1998;7(1):30-40.
32. Yarkoni T, Poldrack RA, Nichols TE, van Essen DC, Wager TD. Large-scale automated synthesis of human functional neuroimaging data. *Nat Methods.* 2011;8(8):665-670.
33. Lacadie CM, Fulbright RK, Rajeevan N, Constable RT, Papademetris X. More accurate Talairach coordinates for neuroimaging using non-linear registration. *NeuroImage.* 2008;42(2):717-725.
34. Vogt BA, Pandya DN, Rosene DL. Cingulate cortex of the rhesus monkey: I. Cytoarchitecture and thalamic afferents. *J Comp Neurol.* 1987;262(2):256-270.
35. Mayberg HS, Liotti M, Brannan SK, *et al.* Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. *Am J Psychiatry.* 1999;156(5):675-682.
36. Zhang X, Melrose AJ, de Santis O, *et al.* Fasting may increase incentive signaling for nonfood rewards. *Nutr Res.* 2020;77:43-53.
37. Dhingra I, Zhang S, Zhornitsky S, *et al.* The effects of age on reward magnitude processing in the monetary incentive delay task. *NeuroImage.* 2020;207:116368.
38. Hanuka S, Olson EA, Admon R, *et al.* Reduced anhedonia following internet-based cognitive-behavioral therapy for depression is mediated by enhanced reward circuit activation. *Psychol Med.* 2022:1-10.