# Supplementary Methods

**Neural food reward processing in successful and unsuccessful weight maintenance.**

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## fMRI task

There were four blocks of reward tasks, consisting of 55 trials each. The block sequence was either SMSM (S=Snacks, M=Money), or MSMS, and was counterbalanced over the participants. The degree of potential rewards varied on three levels as indicated via graphical cues (see supplementary figure S1 for a graphical depiction of the task). In order to guarantee a steady rate of reward vs. non-reward throughout all participants, we used a probabilistic reward pattern, i.e. reward was not paid out in 30 predefined trials (out of the 80 reward trials). In order to increase statistical efficiency, trials were separated by jittered intertrial intervals (ITIs) ranging from 1 to 8 s, with a mean of 3.5 s. An incorrect button press resulted in zero pay-out, a penalty of -1 EUR/ -10 SP was applied if participants failed to react. In the MID task, participants were able to win a maximum of 30 EUR. In the FID task, the maximum amount to be won was 300 SP, with any snack of the basket being 50 SP worth. Before entering the scanner, participants performed a practice version of the task lasting three minutes for each condition for which they received neither payment nor snacks. Additionally, participants performed a response inhibition task following the reward tasks; the results of this task are reported elsewhere. During scanning, participants viewed visual stimuli on a projection screen via a mirror fixed to the head coil.

## fMRI Acquisition parameters

In order to minimize susceptibility artifacts in the orbitofrontal cortex, 30 oblique slices (interleaved acquisition) with a 10° angle relative to the AC-PC axis were acquired with 1-mm interslice gap, using a T2\*-sensitive single-shot EPI sequence with the following parameters: repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, resulting in an in-plane resolution of 3x3x4 mm3, flip angle = 80°, field of view = 192 x 192 mm. Furthermore, high resolution T1 MPRAGE anatomical images were acquired (192 slices, voxel size 1 x 1 x 1 mm3, TR 1570 ms, TE 2.63 ms, 9° flip angle) for anatomical reference.

## fMRI data pre-processing

To account for magnetic field equilibration, four volumes from the beginning of each functional run were excluded from the analyses. Functional images were checked manually for artifacts and corrected for differences in slice acquisition timing. All images were realigned, the allowed motion was limited to ±4 mm translation and ±3 degrees of rotation over the entire experiment, and images were unwarped to correct for artifacts due to susceptibility-by-movement interactions. Individual T1 images were coregistered with the mean T2\* images and subsequently segmented. Both structural and functional images were normalized to the standard anatomical MNI space using the transformation parameters from the segmentation, which resulted in a voxel size of 3 mm3 for the functional images and a voxel size of 1 mm3 for the high-resolution anatomic images. Furthermore, functional images were smoothed with an 8-mm full-width half-maximum isotropic Gaussian kernel. A 128-s high-pass filter was used to remove low-frequency noise and signal drift.

## Region-of-interest analysis

Based on a previous fMRI-study from our group (1) where we employed the same food reward task in a sample of 27 healthy women, we created spheres of 8 mm diameter centered on the peak activation observed in this study during the anticipation of a high compared to no food related reward (expectation of 10 SP versus expectation of 0 SP) in both the right and left ventral striatum (Montreal Neurological Institute (MNI) space: x = 12, y = 2, z = 2, for the right ventral striatum and MNI: x = -9, y = 8, z = -6, for the left ventral striatum). For the analysis of the receipt reward phase we created spheres of 8 mm diameter on the peak activation during the receipt of a food related reward compared to the receipt of no food related reward (receipt of 10 SP versus receipt of 0 SP) in both the right and left orbitofrontal cortex (MNI: x = 42, y = 41, z = -14, for the right orbitofrontal cortex and MNI: x = -39, y = 47, z = -6, for the left orbitofrontal cortex). Due to the common observation of medial orbitofrontal cortex activation during the evaluation of rewards (2), we constructed a sphere of 8 mm diameter centered on the peak of activation (MNI: x = 0, y = 48, z = 6) observed in a previous study where we analyzed neural activation during the receipt of monetary reward (3).

## Procedure

There was a significant difference between glucose, insulin and free fatty acids when comparing hunger and satiety values (ps < 0.05, supplementary table 1), indicating that all participants complied with the fasting instruction. Prior to scanning, participants were presented with a basket containing all the foods they could trade for the SP to be won during the food incentive delay task. During the satiety condition, participants were asked to arrive at the clinic at 9:00 a.m. to complete psychometric evaluations and the SCID interview. Participants filled out a number of self-report and demographic questionnaires and were asked about their eating and dieting behaviours. Specifically, we employed a vocabulary-based test for the assessment of premorbid intelligence (MWT-B, 4), the Eating Disorder Examination Questionnaire (EDE-Q, 5) and the Dutch Eating Behavior Questionnaire (DEBQ; 6). MRI scanning was performed at 12:00 p.m., which corresponded to lunchtime for most of the participants.

**Biochemical analysis of hormonal parameters**

On both days, blood samples were taken shortly before the fMRI measurement (at around 11:45 a.m.) After the blood was centrifuged at 4°C, the serum was separated and stored at −80°C. Glucose concentrations were analysed at the central laboratory of the University of Heidelberg on a Siemens Advia Chemistry XPT system (Erlangen, Germany) using the hexokinase method. The analysis of insulin concentrations were performed at the central laboratory of the University of Heidelberg using the ECLIA insulin immunoassay (Roche, Mannheim, Germany) and the Siemens Advia Centaur XPT system. Leptin concentrations were assessed at the central laboratory of the University of Heidelberg using the Mediagnost Leptin ELISA E07 assay (Reutlingen, Germany) and the Biochrom Anthos 2010 microplate reader (Berlin, Germany) with the Mikrowin 2010 software (Neunkirchen-Seelscheid, Germany). Free fatty acids were analysed at the “MVZ Labor Dr. Limbach & Kollegen GbR” in Heidelberg using the Konelab Prime 60 by Thermo Fisher Scientific (Darmstadt, Germany) and the photometric procedure. Ghrelin values were measured using commercial kits based on a sandwich ELISA assay from Merck Millipore (Darmstadt, Germany) (kit catalogue number: #EZGRT-89K). Immediately after blood collection, Pefabloc-inhibitors were added and serum was acidified to protect the active form of ghrelin. Therefore, the total ghrelin values contain both acyl and des-acyl ghrelin. Each sample was measured in duplicate. The intra-assay coefficients of variation for total ghrelin were 3.4, 4.9 and 6.8% at concentrations of 504.6 and 1938.8µU/ml. The inter-assay coefficients of variations at these concentrations were below 3%. The intra-assay coefficients of variation for active ghrelin were 7.9 and 8.3% at concentrations of 99.8 and 647.9µU/ml, respectively. The inter-assay coefficients of variations at these concentrations were below 14.9 and 3.2%, respectively. Due to technical reasons, the total ghrelin and active values could not be evaluated for 2 participants in the Maintain group during hunger and one Maintain participant during satiety, and total ghrelin values could not be evaluated for one Regain participant during hunger and satiety, active ghrelin values could not be evaluated for one Regain participant during hunger and two Regain participants during satiety.

## References

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**Supplementary Table S1.** Hormonal satiety parameters and hunger rating

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Maintain Group** (mean ± SD) | |  | **Regain Group** (mean ± SD) | |  | |
|  | *Hunger* | *Satiety* |  | *Hunger* | *Satiety* | **Influence of group on satiety-induced changes** |
| Glucose (mg/dl) | 81.9 ± 8.7 | 110.4 ± 19.3\*\*\* |  | 90.9 ± 12.2 | 127.4 ± 28.3\*\*\* | F(1,31)=1.07, p=0.309 |
| Leptin (µg/ml) | 18.3 ± 13.6 | 23.3 ± 15.3\*\* |  | 31.8 ± 13.4 | 39.5 ± 20.2\* | F(1,31)=0.63, p=0.431 |
| Insulin (mU/ml) | 9.2 ± 5.7 | 66.9 ± 31.2\*\*\* |  | 13.9 ± 11.2 | 96.3 ± 48\*\*\* | F(1,33)=1.91, p=0.177 |
| Free fatty acids (mmol/l) | 0.8 ± 0.4 | 0.3 ± 0.3\*\* |  | 0.94 ± 0.3 | 0.4 ± 0.2\*\*\* | F(1,33)=0.18, p=0.672 |
| Total Ghrelin (fmol/ml)† | 514.3 ± 131.6 | 412.5 ± 105.6\*\* |  | 655.8 ± 339.8 | 547.3 ± 246.8 | F(1,28)=0.01, p=0.909 |
| Active Ghrelin (fmol/ml)§ | 363.4 ± 136.3 | 241.1 ± 107.7\*\* |  | 449.6 ± 279.8 | 365.2 ± 188.3 | F(1,27)=0.35, p=0.558 |
| Hunger rating *prior measurement* | 5.9 ± 2.6 | 0.6 ± 1.1\*\*\* |  | 4.9 ± 3.2 | 0.6 ± 0.9\*\*\* | F(1,31)=0.97, p=0.333 |
| Mood rating *prior measurement* | 6.5 ± 1.7 | 7.9 ± 1.8\*\*\* |  | 6.7 ± 1.4 | 6.7 ± 1.7 | *F(1,31)=9.19, p=0.005* |
| Money won during the MID task | 28.6 ± 1.6 | 28.5 ± 1 |  | 28.5 ± 2.2 | 28.7 ± 1.9 | F(1,29)=0.63, p=0.431 |
| SP won during the FID task | 292.6 ± 10 | 285.4 ± 14.5\* |  | 278.5 ± 30 | 280.5 ± 21.2 | F(1,30)=0.19, p=0.662 |

Differences between satiety conditions assessed using two-tailed paired t-tests: \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001, Differences between groups assessed using a repeated measures ANOVA with group as a between factor and satiety state as the repeated/within factor. †: Data missing for total Ghrelin for two participants of the Maintain group during hunger and one participant of the Maintain group during satiety, data missing for one participant of the Regain group during hunger and one participant of the Regain group during satiety. §: Data missing for active Ghrelin for two participants of the Maintain group during hunger and one participant of the Maintain group during satiety, data missing for one participant of the Regain group during hunger and two participants of the Regain group during satiety. MID: Monetary Incentive Delay task, FID: Food Incentive Delay task, SP: Snack Points.

F:\NSM\11Paper_CON\11Nature_neuroscience_paper_CON\Abbildungen\Figure1_neu.tif**Supplementary Figure S1:** Graphical depiction of the FID and MID tasks. Trials begin with a “cue” indicating the amount of money or SPs the participants can win when reacting correctly during the subsequent discrimination task (anticipation phase). Following target presentation, participants are informed about the amount of money or number of SPs won during the respective trial and their cumulative total winnings thus far (i.e. the receipt of reward phase). The MID task employs graphical depictions of a wallet filled with EUR 1, EUR 0.20 or EUR 0, corresponding to the amount of money won during the trial. The FID task uses pictures of either a large, small or empty basket, depending on the number of SPs won during the trial.



**Supplementary Figure S2:** Masks used to extract percent signal change of BOLD activation from the right (A) and left (D) ventral striatum. In overweight participants with *successful* weight maintenance, activity in the right (B) and left (E) ventral striatum was related to reward level (*F(*1,16)=5.54, p=0.009 and *F(*1,16)=5.68, p=0.008, respectively) during the expectation of monetary related reward. There was no influence of satiety status (ps>0.561). In overweight participants with *unsuccessful* weight maintenance, activity in the right (B) and left (E) ventral striatum was related to reward level (*F(*1,14)=7.02, p=0.003 and *F(*1,14)=6.54, p=0.005, respectively) during the expectation of monetary related reward. There was no influence of satiety status (ps>0.247). Error bars depict SEM.



**Supplementary Figure S3:** Masks used to extract percent signal change of BOLD activation from the right (A), left (D) and medial (G) orbitofrontal cortex. In overweight participants with *successful* weight maintenance, activity in the right (B) and left (E) OFC was not related to reward level (ps>0.375) or satiety state (ps>0.366). Activity in the medial OFC (H) was related to reward level (*F(*1,16)=4.25, p=0.023) but not to satiety state (p=0.717). In overweight participants with *unsuccessful* weight maintenance, activity in the right (B), left (E) and medial (H) OFC was not related to reward level (ps>0.346) or satiety state (ps>0.443). Error bars depict SEM.