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The effect of hunger state on hypothalamic functional connectivity in response to food cues

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Abstract

The neural underpinnings of the integration of internal and external cues that reflect nutritional status are poorly understood in humans. The hypothalamus is a key integrative area involved in short- and long-term energy intake regulation. Hence, we examined the effect of hunger state on the hypothalamus network using functional magnetic resonance imaging. In a multi-center study, participants performed a food cue viewing task either fasted or sated on two separate days. We evaluated hypothalamic functional connectivity (FC) using psychophysiological interactions (PPI) during high versus low caloric food cue viewing in 107 adults (divided into four groups based on age and body mass index (BMI); age range 24 to 76 years; BMI range 19.5 to 41.5 kg/m^2). In the sated compared to the fasted condition, the hypothalamus showed significantly higher FC with the bilateral caudate, the left insula and parts of the left inferior frontal cortex. Interestingly, we observed a significant interaction between hunger state and BMI group in the dorsolateral prefrontal cortex (DLPFC). Participants with normal weight compared to overweight and obesity showed higher FC between the hypothalamus and DLPFC in the fasted condition. The current study showed that task-based functional connectivity of the hypothalamus can be modulated by internal (hunger state) and external cues (i.e. food cues with varying caloric content) with a general enhanced communication in the sated state and obesity-associated differences in hypothalamus to DLPFC communication. This could potentially promote overeating in persons with obesity.

Introduction

As obesity rates keep increasing dramatically around the globe, actions are needed to support healthy diets and life-styles. In our obesogenic environment, we are continuously exposed to highly appetizing food cues, which can promote overconsumption and weight gain (Ferriday and Brunstrom, 2008). Brain reactivity to food cues is associated with portion size selection (Hege et al., 2018; Veit et al., 2020), food choice and food consumption (Christensen et al., 2021; Stice et al., 2008) and is even predictive for the outcome of weight-loss interventions (Hermann et al., 2019; Stice and Yokum, 2018).

Specifically, in neuroimaging studies, food cue reactivity (FCR) tasks are used to examine brain responses to food images depending on caloric content, palatability, healthiness, macronutrient content or level of processing (Smeets et al., 2019). In healthy individuals, food images activate the so-called appetitive brain network, which includes the amygdala, striatum, insula and the orbitofrontal cortex (OFC) (Neseliler et al., 2017). High compared to low caloric foods elicit differential activation in the ventral striatum, hypothalamus, the left frontal and occipital cortex and the right inferior temporal region. These areas are involved in reward processing and homeostasis but also in visual processing and executive function (Neseliler et al., 2017). Persons with obesity respond to palatable food cues with heightened FCR in brain regions important for reward and gustatory processing (Christensen et al., 2021; Devoto et al., 2018; Kenny, 2011; Pursey et al., 2014; Stice et al., 2008). In contrast, brain regions important for cognitive control have been found to be less responsive in persons with obesity (Brooks et al., 2013; Christensen et al., 2021; Han et al., 2018). However, this view of heightened incentive salience of food cues in obesity was recently challenged. A meta-analysis, by Morys et al. (2020) failed to identify consistent differences in FCR between normal weight and overweight/obese groups. These findings point to additional factors that could mediate the FCR activation pattern. In particular, sex, age, hunger state, body mass index (BMI) and food stimulus type may significantly influence FCR activation patterns (Bennett et al., 2021; Charbonnier et al., 2018; Smeets et al., 2019; Wever et al., 2021). With increasing age, the insular cortex is less responsive to food cues, while FCR in the fusiform gyrus, a higher visual area, showed stronger activity with increasing age (Morys et al., 2020).

Hunger can lead to increased reactivity to food cues in primary and higher level visual processing areas in normal weight individuals (Charbonnier et al., 2018; Fuhrer et al., 2008; Siep et al., 2009; Uher et al., 2006). Moreover, in a fasted state, high opposed to low-caloric food images elicit stronger activity in the appetitive brain network (Goldstone et al., 2009; Mehta et al., 2012; van der Laan et al., 2011). Persons with obesity tend to show higher FCR when sated in areas involved in executive function, reward and emotional processing compared to lean counterparts (Devoto et al., 2018; Pursey et al., 2014), particularly in the ventral striatum (Devoto et al., 2018).

Relatively little is known about the role of the hypothalamus in response to visual food-cues. The hypothalamus is a key integrative area involved in the control of basic life functions including short- and long-term energy intake regulation. Distinct nuclei in the hypothalamus are involved in the control of food intake with specific roles in the processing of hunger and satiation (Berthoud and Munzberg, 2011; Hetherington and Ranson, 1942). These include the ventromedial, the lateral, and the dorsomedial hypothalamus as well as the arcuate and paraventricular nuclei (Nieuwenhuys et al., 2008). In humans, resting state fMRI studies showed that the hypothalamus is functionally coupled to the regions of the appetitive brain network (Kullmann et al., 2014; Kullmann and Veit, 2021). Particularly the influence of fasting has been investigated on hypothalamus resting-state functional connectivity showing higher functional connectivity to the medial PFC (Lips et al., 2014; van de Sande-Lee et al., 2011; Wijngaarden et al., 2015; Wright et al., 2016) and insula cortex (Lips et al., 2014; Wijngaarden et al., 2015) in persons of normal weight. An overall reduction in hypothalamic functional connectivity were observed in the sated compared to the fasted state (Lips et al., 2014; Sewaybricker et al., 2020). This effect was less pronounced in persons with overweight and obesity (Lips et al., 2014).

With the current study, we investigated task-based functional connectivity of the hypothalamus network by using high and low-caloric food images in a fasted and sated condition in males and females of different age and weight groups. We hypothesized that hypothalamic functional connectivity is higher with reward and gustatory related regions in the fasted compared to the sated state during the visual food cue reactivity task (high-caloric minus low-caloric food images). In normal weight participants, we expected higher FC between the hypothalamus and the DLPFC, while participants with overweight/obesity were expected to show higher hypothalamus FC to reward related brain region, particular in the fasted state. In the elderly group, we hypothesized a decline in hypothalamic FC independent of hunger state. In exploratory analyses, we investigated associations between hypothalamic FC and peripheral insulin resistance as well as experienced fullness and hunger.

Methods

Participants

As part of the European Full4Health project (https://www.abdn.ac.uk/rowett/research/full4health.php) functional MRI measurements were performed in three different countries (The Netherlands, Scotland and Greece). In the present study, we included healthy adults of normal-weight (BMI 20-25 kg/m^2), overweight and obesity (BMI ≥ 27.5 kg/m^2) with two different age ranges (adult: 20 -50 years, elderly: > 60 years. Participants were categorized into four groups based on their body mass index (BMI) and age (normal-weight adults, normal-weight elderly, overweight/obese adults and overweight/obese elderly). Only right-handed, non-smoking participants without major weight fluctuation (± 5 kg) in the last 6 months were included in the study. Medication (except aspirin/paracetamol and oral contraceptives and anticoagulants and cholesterol medication in elderly) and no excessive alcohol consumption (> 28 units per week) was allowed. Furthermore, participants with disturbed eating behavior (measured with Dutch Eating Behavior Questionnaire (van Strien et al., 1986)) food allergies, special diets, eating disorders as well as metabolic syndrome, endocrine disease and gastrointestinal disorders were excluded. Moreover, the participants had to fulfill the inclusion criteria for MRI measurements (e.g. no metal implants).

A total of 133 participants were enrolled in the study. After data quality control (see below), 107 participants (57 women, 50 men; BMI range 19.5 – 41.5 kg/m^2; age range 24.2 – 76.1 years) were included in the final data analyses (see Table 1 for participants' characteristics). The study was registered at NTR (trialregister.nl).

Study procedures

After an overnight fast, participants came on two separate mornings for an MRI scanning session (sated and fasted condition). The MRI sessions were counterbalanced and separated by 1-2 weeks. In the sated condition, participants were scanned 1 hour after consumption of a fixed amount of liquid meal (for details see (Charbonnier et al., 2018)). On both days, a blood sample was taken followed by a food cue reactivity task in the MRI scanner. Hunger and fullness were rated at baseline after an overnight fast, at 30 minutes (after liquid breakfast or no breakfast) and at 55 minutes (before the food viewing task) using 9-point Likert scales. For more details on study design see recent publications (Charbonnier et al., 2018; Wever et al., 2021).

Food cue viewing fMRI task

The food cue reactivity task consisted of 6 blocks with high-caloric food (HC), 6 blocks with low-caloric food (LC) and 6 blocks with non-food (NF) items. Each block included 7 images and lasted for 20.5 seconds, the inter-block interval varied between 3.5 and 4 s. The order of the blocks was kept constant over the course of the task (LC, NF, HC). The images were selected from a standardized image set (Charbonnier et al., 2016) and were adapted to ensure recognizability and liking in each of the three countries. The imaging viewing task lasted 442 seconds in total. At the beginning of the experiment, the participants received the following task instruction: “In the next task you will see food and non-food products. Please look at the images and pay close attention, since at the end of the MRI session you will be asked a couple of questions regarding the images shown during this task.” After the MRI session, participants were shown 10 images for which they had to indicate whether they had seen them during the task. See (Charbonnier et al., 2018) for more details.

Image acquisition and processing

Scanning was performed on a Philips Achieva 3.0 T MRI scanner (Philips Healthcare, Best, NL) in all three countries. Functional images were obtained with an 8-channel SENSE head-coil using a 2-D echo planar imaging (EPI) sequence with the following parameters: voxel size 4 mm isotropic; repetition time (TR) = 1400 ms; echo time (TE) = 23 ms; flip angle = 70°; 30 axial slices; SENSE-factor = 2.4 (anterior-posterior). 316 functional images were acquired. A high-resolution anatomical image (T_1-weighted scan) was acquired at 1 x 1 x 1 mm^3 resolution.

Image preprocessing

Spatial and temporal preprocessing and statistical analyses were carried out with SPM12 (http://www.fil.ion.ucl.ac.uk/spm). Slice timing correction and realignment was performed for each fMRI timeseries, and the structural scan was coregistered to the mean functional image. The T1 weighted anatomical image was segmented using unified segmentation, and normalization parameters were estimated. The elderly group did not show any apparent brain abnormalities or damages, hence, we used a common template created using DARTEL for all participants, which is considered more robust for normalization. The template was used to normalize the functional scans to MNI space. The data were then smoothed with an isotropic 8-mm full width at half maximum Gaussian kernel. The ArtRepair toolbox (http://cibsr.stanford.edu/tools/ArtRepair/ArtRepair.htm) was applied to detect and repair anomalously noisy volumes. Volumes that moved more than 1 mm/TR were repaired.

Subject level analyses

For each participant, a design matrix was created with the regressors high-caloric food, low-caloric food and non-food, separately for the two conditions (fasted/sated). Each regressor was convolved with a canonical hemodynamic response function. Data were high-pass filtered with a cutoff of 128 s and low pass filtered (Autoregression Model AR(1)). The following contrasts were created: high caloric minus low caloric food images, food images (high and low caloric combined) minus non-food images.

A visual inspection of the activation maps (T-maps) during image viewing revealed datasets with minimal or no activation. Therefore, we adopted a specific quality criteria, which was already applied in the same multi-center study (Wever et al., 2021). We calculated the number of voxels with significant brain activity at $p<0.001$ in a composite mask including the fusiform gyrus, lingual gyrus, occipital gyrus and extended visual cortex based on the AAL atlas, separately for the two days. Only participants that exceeded the 10 % percentile of active voxels (cut off: 224 voxels) on both days were included in the analysis. A total of 21 participants were below this threshold on one or two visits and five participants were excluded based on incomplete brain coverage during fMRI recording. The final sample of 107 participants had 853 ± 326 significant voxels in the visual cortex ROI.

Generalized Psychophysiological Interaction (gPPI)

A generalized PPI analysis (McLaren et al., 2012) (ppi version 13.1) was conducted to investigate task related functional connectivity of the hypothalamus with other brain regions, for each measurement day (fasted/sated). We generated a mask of the hypothalamic nuclei related to energy regulation using the Neudorfer hypothalamus atlas (Neudorfer et al., 2020) including the lateral hypothalamus, the ventromedial hypothalamus, the dorsomedial hypothalamus, the arcuate and the paraventricular nuclei. First, we created a combined mask of the regions and then resampled the masks to the dimension and voxel size of the Automated Anatomical Labelling atlas 3 (AAL3 (Rolls et al., 2020) dimensions: 91x109x91, voxel size: 2x2x2 mm^3). In next step, we resampled the mask to a voxel size of 4 mm^3. Using these approach a total of 3*8 voxels covered this mask (Figure 1).

Opposed to the traditional PPI approach the generalized PPI allows the integration of several interaction vectors in the design matrix. The time-series for each seed region was extracted using the first eigenvariate. To adjust for non-task regressors an omnibus F-test was performed in the original first level analysis. This allows removal of non-neural sources (e.g. motion) before deconvolution of the signal. In a next step, for each condition a PPI interaction term was created (high caloric food, low caloric food, nonfood). The inclusion of all conditions in the design matrix allows a better estimate of the underlying psychophysiological interactions. Finally, a contrast vector was created testing the effect of caloric content (high caloric minus low caloric food images).

Second-level group analysis

The resulting contrast images (high minus low-caloric food) were entered into a second level full-factorial model with the within-subject factor: hunger condition (fasted vs. sated), and between subject-factor: BMI group (lean vs. overweight/obese) and age group (adult vs. elderly). As covariates of no interest, the country site (dummy coded with two regressors) and sex (female/male) were included in the design matrix.

In a separate exploratory analysis, we investigated the effect of sex as additional categorical factor. Here we used age as a continuous covariate in the design matrix. The factors hunger condition and BMI group were the same as in the above mentioned analyses.

A statistical threshold of $p < 0.001$ uncorrected and a $p<0.05$ family wise error (FWE) corrected for multiple comparisons at a cluster level was applied. We used the SPM Cluster Threshold toolbox (https://github.com/CyclotronResearchCentre/SPM_ClusterSizeThreshold) to compute the minimum number of voxels determining a significant cluster. Significant activations that survived FWE correction at a peak level ($p_{FWE}<0.05$) were also reported. Additionally, small volume correction was performed for the striatum (putamen, pallidum, caudate), insula and the DLPFC, as they are *a priori* regions of interest due to their involvement in food-cue processing (Neseliler et al., 2017). The masks were based on the automated anatomical labeling atlas 3 (https://www.oxcns.org) and the *wfu* pick atlas (http://fmri.wfubmc.edu/software/PickAtlas) (Maldjian et al., 2003). For the DLPFC, we used a mask created by Matthjis Vink (https://matthijs-vink.com/my-open-science). Correction for multiple comparisons for small volume correction was restricted to the masks (Bonferroni corrected for the number of ROI's; corrected threshold $p= 0.016$).

To investigate to which extent other important factors as experienced hunger and fullness and markers of peripheral insulin resistance (HOMA-IR) modulated the FC pattern, the connectivity parameters of significant clusters were extracted for additional correlation analyses.

Results

gPPI analyses of the hypothalamus network in response to high compared to low caloric food cues

Hypothalamus functional connectivity network

We observed higher functional connectivity between the hypothalamus and the bilateral caudate in the sated compared to the fasted state (Figure 2 A). The cluster of the left caudate extended into the left insula, the left frontal inferior triangularis and the left frontal inferior operculum. There was a significant BMI group x hunger state interaction in the right DLPFC, the left middle occipital gyrus and the left precentral gyrus (Table 2). Post hoc contrasts showed that participants with normal weight compared to participants with overweight/obesity exhibited stronger FC between hypothalamus and the right DLPFC (Figure 3), left insula and middle occipital gyrus in the fasted but not in the sated state (Table 2, Figure 2B). There was no main effect of age group. There were no 2-way interactions between age group and hunger state and no significant 3-way interaction.

Effect of sex on hypothalamic functional connectivity

In exploratory analyses, there was no main effect of sex or interaction with hunger state or BMI group in the full factorial analyses adjusted for age (all $p>0.05$).

Correlation analyses of hypothalamus network with fullness and hunger ratings

For the sated day, the hypothalamus to right caudate connection significantly correlated with the change in fullness ratings (from before to after the liquid meal) adjusted for sex, age and BMI ($r= -0.272$, $p_{adj}= 0.009$). Hence, participants showing an increase in experienced fullness show lower functional connectivity between the hypothalamus and caudate on the sated day.

Correlation analyses of hypothalamus network with HOMA-IR

The differential (sated minus fasted condition) functional connectivity between the hypothalamus and left caudate correlated with HOMA-IR ($r= 0.214$, $p= 0.032$ adjusted for sex and age). Hence, participants with insulin resistance showed stronger FC between the hypothalamus and the caudate in the sated compared to the fasted state in response to high vs. low caloric food cues.

General linear model and gPPI of food cue reactivity (based on high minus low caloric food cue contrast)

In order to compare our FCR results with previous findings, a second level group analysis was carried out using the high minus low caloric contrast to evaluate changes in regional task-related brain activity. We found stronger activations in the caudate, insula, anterior cingulate, orbitofrontal cortex for high minus low caloric food stimuli (supplementary table 1 and supplementary figure 1) with higher activity in the putamen, caudate and pallidum in the fasted compared to the sated state. Additional gPPI analyses were carried out to investigate task-related functional connectivity based on the reported high minus low caloric activity changes in the FCR task (using the seeds: left caudate, right caudate, left insula, left ACC). Overall, we found higher task-based functional connectivity in the caudate, insula, anterior cingulate, and orbitofrontal cortex in the sated compared to the fasted state (for more information see supplementary material).

Discussion

The aim of the current study was to investigate task based functional connectivity patterns of the hypothalamus in the sated compared to the fasted state in response to food images with varying caloric content. When viewing high compared to low caloric food cues, we could confirm higher activity in the insula, anterior cingulate, orbitofrontal cortex and caudate, as previously reported (Neseliler et al., 2017). Based on previous studies, we postulated that task-based hypothalamic functional connectivity would be higher in the fasted state during the evaluation of high caloric food cues. Contrary to our hypothesis, we found higher hypothalamic functional connectivity, in the sated compared to the fasted state, with brain regions involved in reward and gustatory processing and executive function. However we found a significant interaction between BMI status and hunger state in the right DLPFC. The coupling between hypothalamus and right DLPFC was strongest in persons with normal weight compared to persons with overweight/obese in the fasted state. As hypothesized, peripheral metabolism and subjective ratings of fullness correlated with hypothalamic functional connectivity.

We observed higher functional connectivity between the hypothalamus to striatal regions, and primary and secondary gustatory cortices in the sated condition. Functional coupling between the homeostatic regions of the brain and reward and taste processing areas was strengthened after a meal when evaluating high caloric in comparison to low caloric food cues. Concurrently, glucose, sucrose and fat ingestion increased resting-state functional connectivity of the hypothalamus to the striatum (Page et al., 2013) and insular cortex (Frank-Podlech et al., 2019; Kilpatrick et al., 2014). In the current study, the hypothalamus network showed the greatest differences in functional coupling between the sated and the fasted state. Sensory information such as visual food cues and internal signals from the periphery reflecting the current hunger state have been postulated to converge in the insula and higher cortical areas and to influence food choice and eating behavior (de Araujo et al., 2020). In our study, satiation strengthened the hypothalamic functional connections in response to high caloric food images. This could indicate that in the sated state communication is enhanced in the hypothalamus network specifically to the striatum and the gustatory cortex (i.e. insula and frontal operculum). Independent of nutritional state, a recent systematic review on resting-state fMRI showed higher hypothalamus functional connectivity to reward brain regions and lower functional connectivity to cognitive regions in persons with obesity (Syan et al., 2021). Concomitantly, in the current study, the hypothalamus connections particularly to the caudate nucleus was affected by experienced fullness and metabolic health showing stronger functional coupling in persons with greater insulin resistance (based on HOMA-IR). This corresponds with FCR studies showing an increased activation of striatal regions with higher HOMA-IR values (Drummen et al., 2019; Jastreboff et al., 2013). Moreover, insulin is thought to play a pivotal role in brain reward regulation by modulating dopamine function in the striatum (Kullmann et al., 2021), thereby decreasing food palatability ratings and food intake (Kullmann et al., 2015; Tiedemann et al., 2017). In people with insulin resistance, insulin action in the dopaminergic circuitry is disturbed, which is related to higher preference for palatable foods (Kullmann et al., 2020). Hence, disturbances in reward function may lead to overeating and facilitate the transition from obesity-associated insulin resistance to the development of type 2 diabetes.

Hypothalamus connectivity with cognitive regions (i.e. the DLPFC) was affected by weight status in the hungry but not sated state. Adults and elderly of normal weight showed higher hypothalamus to DLPFC connectivity when viewing high caloric compared to low caloric food cues. Similarly, Charbonnier et al. (2018) showed greater DLPFC activity to high versus low caloric cues in the fasted compared of sated state independent of age. DLPFC recruitment is vital for healthy food choices and dietary self-control (Hare et al., 2009; Hare et al., 2011; Kohl et al., 2019; Lowe et al., 2019; van Meer et al., 2019; van Meer et al., 2017). In persons with overweight/obesity, previous studies have shown diminished DLPFC activity in response to food cues (Brooks et al., 2013; Christensen et al., 2021; Veit et al., 2021) as well as reduced hypothalamus to DLPFC connectivity (Syan et al., 2021). Internal and external signals can facilitate an increase in hypothalamic functional connectivity to the PFC. The hormonal satiety signal insulin (Kullmann et al., 2017) and the choice of healthy food items enhanced hypothalamus PFC connectivity (Harding et al., 2018). Whether higher hypothalamic functional connectivity to the DLPFC is linked to better dietary self-control is currently not known. Future studies need to evaluate whether the hypothalamus network that responds to high caloric food cues can predict food choice behavior, food consumption or metabolism.

Limitations

Our study sample included a large BMI range, but due to the limited sample size, we were not able to evaluate whether persons with overweight versus obesity may show separable hypothalamus FC patterns. It is currently not clear whether our findings can be generalized to resting-state functional connectivity patterns. Recent studies investigating the interplay of the hypothalamus under different nutritional states and hormonal manipulations primarily used resting-state fMRI (Kullmann and Veit, 2021). Only few studies examined task-evoked and task-independent resting-state functional connectivity within the same sample (Donofry et al., 2020; Lynch et al., 2018; Mehl et al., 2019), however with different seed regions of the hypothalamus. Donofry et al. (2020) investigated food-cue induced and resting-state functional connectivity showing a dissociable pattern in persons with overweight and obesity. Higher BMI was associated with weaker functional connectivity during rest and higher functional connectivity in response to high caloric food cues (Donofry et al., 2020). This suggests context-dependent functional connectivity among individuals who are overweight and obese. In line with this contextualization, there is evidence that engaging in a task may suppress resting-state functional connectivity (Lynch et al., 2018). Hence, hypothalamus communication during resting-state state may lead to different results in the sated compared to the fasted state as observed in the current study. Further studies are needed to disentangle internal and externals cue integration on the hypothalamic functional connectivity profile during task-based and resting-state fMRI.

Conclusion

The current study shows that brain functional connectivity is modulated by internal as well as external cues. While viewing high caloric foods, hypothalamic functional connectivity to reward and cognitive regions of the brain was higher in the sated state. Lower functional coupling to the prefrontal cortex was observed in participants with obesity. This could potentially promote overeating and accelerate the transition to metabolic diseases as type 2 diabetes. Whether hypothalamus connectivity profiles are related to overeating and weight gain still needs to be investigated.

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Authors' Contributions

SK: Conceptualization; Formal analysis; Writing - original draft; Writing - review & editing. **RV**: Formal analysis; Methodology; Roles/Writing - original draft; Writing - review & editing. **DC**: Investigation; Writing - review & editing. **WB**: Investigation; Writing - review & editing. **OA**: Investigation; Supervision; Writing - review & editing. **AJ**: Funding acquisition; Resources; Investigation; Supervision; Writing - review & editing. **YM**: Funding acquisition; Resources; Investigation; Supervision; Writing - review & editing. **HP**: Funding acquisition; Resources; Investigation; Supervision; Writing - review & editing. **PS**: Funding acquisition; Conceptualization; Resources; Investigation; Methodology; Project administration; Supervision; Roles/Writing - original draft; Writing - review & editing; Data curation.

Ethics statement

The study was approved in Utrecht by the UMC Medical Ethical Committee, in Aberdeen by the National Health Service North of Scotland Research Ethics Service and in Athens by the Bioethics Committee of Harokopio University and the Greek Ministry of Education. The study was registered in the Dutch trial registry (www.trialregister.nl; NL3512/NTR3644).

Patent consent statement

All participants provided written informed consent.

Data availability statement

The authors have documented all data, methods, and materials used to conduct this research study, and anonymized data will be shared by request from any qualified investigator.

Table 1. Participant characteristics

		FEMALE	MALE		
SEX (COUNT)	Adult NW	16	13		
	Elderly NW	15	10		
	Adult OW	11	16		
	Elderly OW	15	11		
	all	57	50		
		mean	***STD***	***Min***	***Max***
AGE IN YEARS	Adult NW	33.41	6.92	25.03	47.25
	Elderly NW	70.04	3.11	64.96	76.11
	Adult OW	35.26	6.69	24.21	45.85
	Elderly OW	67.31	3.16	61.95	74.88
	all	50.67	18.07	24.21	76.11
BODY MASS INDEX (KG/M^2)	Adult NW	22.95	1.85	19.74	26.10
	Elderly NW	23.51	1.98	19.52	26.58
	Adult OW	30.99	3.27	27	41.49
	Elderly OW	31.35	2.92	26.85	36.29
	all	27.11	4.73	19.52	41.49
HOMA-IR	Adult NW	1.38	0.80	0.44	4.04
	Elderly NW	1.68	1.10	0.30	5.33
	Adult OW	3.72	2.16	1.11	10.54
	Elderly OW	3.97	1.93	1.30	10.55
	all	2.67	1.96	0.30	10.55

Table 2. Hypothalamus functional connectivity network in response to high versus low caloric food cues.

Brain region	Hemi	MNI Coordinates			Peak- t	p_{FWE}
		x	y	z		
Sated > Fasted						
Caudate	L	-16	12	20	4.94	<0.001
Caudate	R	16	4	24	3.82	0.027^{SVC}
Frontal Inferior Triangular part	L	-32	36	12	4.76	<0.001
Insula*	L	-32	20	12	4.82	0.013
Precentral gyrus/Frontal Inferior operculum	L	-56	8	28	3.87	0.043
Fasted > Sated						
No differential activation	--	--	--	--	--	--
Overweight/obese versus normal weight						
No differential activation	--	--	--	--	--	--
Interaction hunger x BMI status						
Superior frontal (DLPFC)	R	20	28	40	4.54	0.039
Precentral gyrus	L	-36	-12	36	4.07	0.002
Middle occipital	L	-24	-96	4	4.04	0.006
Post-hoc contrasts						
Normal weight > overweight/obese hungry state						
Superior frontal (DLPFC)	R	20	28	40	4.42	0.004^{SVC}
Insula	L	-28	28	12	4.37	<0.001
Frontal Inferior triangularis (DLPFC)	R	44	20	24	3.91	0.024^{SVC}
Middle occipital	L	-40	-64	8	3.78	0.022
Normal weight < overweight/obese sated state						
No differential activation	--	--	--	--	--	--
Normal weight < overweight/obese hungry state						
No differential activation	--	--	--	--	--	--
Normal weight > overweight/obese sated state						
	--	--	--	--	--	--

** the insula is part of the same cluster as the frontal inferior triangularis, but shows also significant differential connectivity on a peak level ($p<0.05$ FWE corrected). Hemi = Hemisphere, L = left, R = right; p value FWE corrected using whole-brain cluster correction, the cluster size threshold for the analyses was 47 voxels; SVC p_{FWE} small volume corrected for ROIs.*

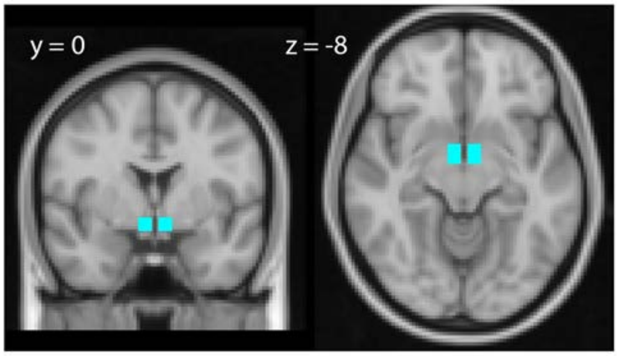

Figure 1: Hypothalamus mask applied in the PPI analysis overlaid on a study-specific T1 weighted image in MNI space. Hypothalamus mask was created based on the Neudorfer hypothalamus atlas including the lateral hypothalamus, the ventromedial hypothalamus, the dorsomedial hypothalamus, the arcuate and the paraventricular nuclei.

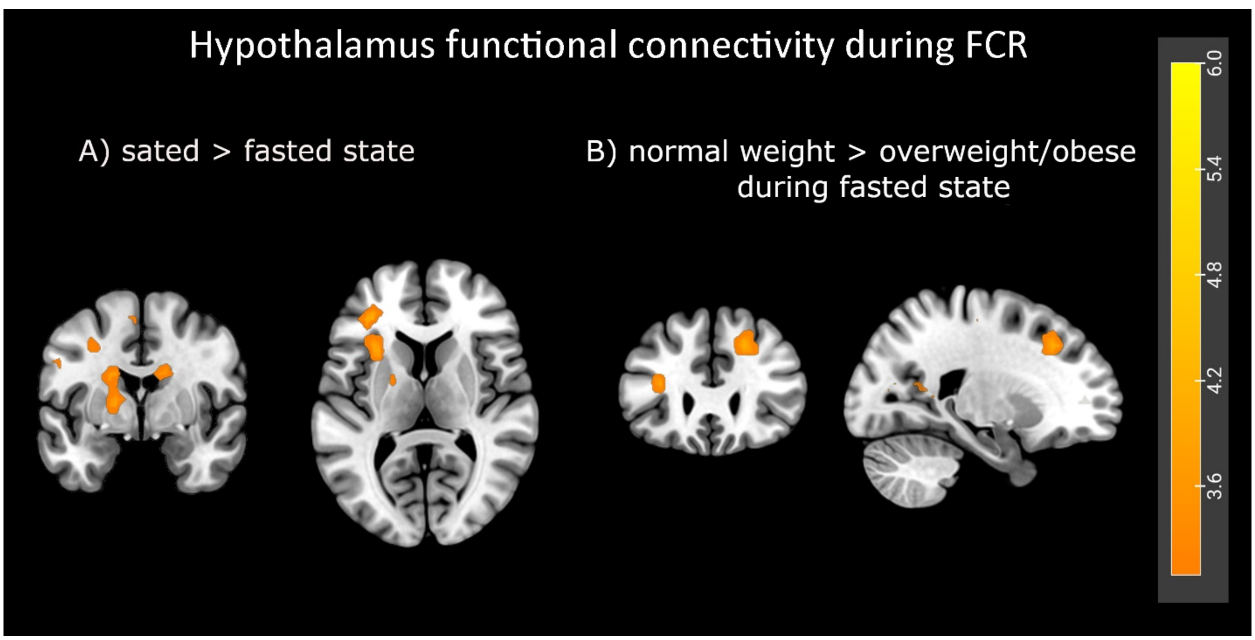

***Figure 2**. Hypothalamus task-based functional connectivity network. A) Shown are clusters of the hypothalamus network revealing higher functional connectivity in the sated versus the fasted state. B) Shown are clusters of the hypothalamus network revealing higher functional connectivity in normal weight in comparison to overweight/obese* participants *during the fasted state. Color maps correspond to t-values (thresholded at t=3.13 / p<0.001 uncorrected for display).*

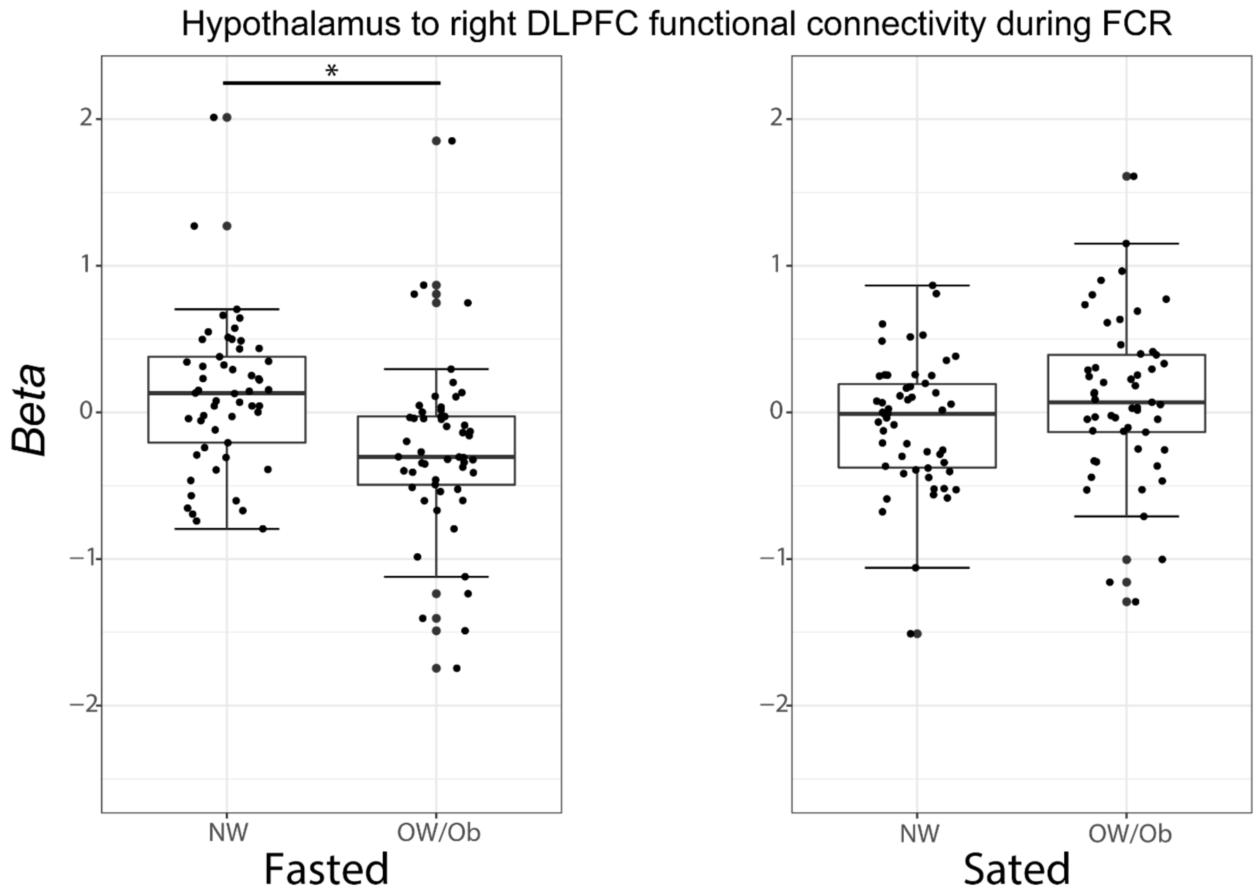

Figure 3. *Box plot showing hypothalamic functional connectivity to the right dorsolateral prefrontal cortex (DLPFC) in the fasted and sated state during food cue reactivity (FCR) in normal-weight (NW) and overweight/ obese (OW/Ob) participants. A 2-way interaction between hunger state and BMI group was identified in parts of the right DLPFC (pFWE<0.05). In the fasted state, NW participants showed higher functional connectivity between the hypothalamus and the right DLPFC than the OW/Ob group. No group differences were observed in the sated state.*

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