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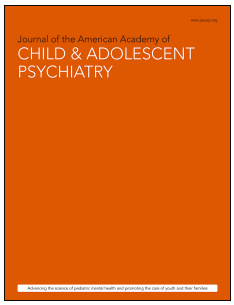

Hypothalamic Networks in Adolescents With Excess Weight: Stress-Related Connectivity and Associations With Emotional Eating

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PII: S0890-8567(18)31981-6

DOI: https://doi.org/10.1016/j.jaac.2018.06.039

Reference: JAAC 2401

To appear in: *Journal of the American Academy of Child & Adolescent Psychiatry*

Received Date: 16 October 2017

Revised Date: 7 June 2018

Accepted Date: 18 June 2018

Please cite this article as: Martín-Pérez C, Contreras-Rodríguez O, Vilar-López R, Verdejo-García A, Hypothalamic Networks in Adolescents With Excess Weight: Stress-Related Connectivity and Associations With Emotional Eating, *Journal of the American Academy of Child & Adolescent Psychiatry* (2018), doi: https://doi.org/10.1016/j.jaac.2018.06.039.

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RH = Hypothalamic Networks in Adolescence

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Editorial
Clinical Guidance
Supplemental Material

Accepted June 20, 2018

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This study has been funded by project grant NEUROCOBE (HUM-6635) from the Andalusian Council of Innovation, Science and Industry, Spain. Dr. Contreras-Rodríguez is funded by postdoctoral “PERIS” contract (SLT006/17/00236) Catalan Government, Spain.

Disclosure: Drs. Martín-Pérez, Contreras-Rodríguez, Vilar-López, and Verdejo-García report no biomedical financial interests or potential conflicts of interest.

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Abstract

Objective: Adolescents with excess weight are particularly sensitive to stress, which may contribute to the presence of emotional eating behaviors. It is proposed that this may be due to alterations in the connectivity between hypothalamic networks and regions of the "emotional nervous system", involved in the regulate on of energy balance and stress processing. However, this remains to be clarified in adolescents with excess weight.

Method: We investigated whole-brain differences in the functional connectivity of the medial and lateral hypothalamus (MH and LH) between adolescents with excess (EW, N=53; mean age: 14.64, SD=1.78) and normal weight (NW, N=51; mean age=15.29, SD=1.75) using seed-based resting-state analyses. Then, in a subset of 22 adolescents with EW (mean age=15.75, SD=1.70) and 32 with NW (mean age=15.27, SD=2.03), we explored for group interactions between the MH/LH networks and stress response in the Trier Social Stress Task (TSST), and emotional eating, assessed with the Dutch Eating Behavior Questionnaire (DEB-Q).

Results: Compared to NW, EW showed higher functional connectivity in the LH-orbitofrontal cortex, ventral striatum, anterior insula, and in the MH-middle temporal cortex networks. EW also showed lower connectivity in the LH-cerebellum, and in the MH-middle prefrontal, pre and postcentral gyri networks. In EW, higher connectivity of the LH-nucleus accumbens and LH-midbrain networks were associated with stress response. Higher connectivity in the LH-midbrain was also associated with a greater presence of emotional eating behaviors within EW.

Conclusion: Adolescents with EW showed functional connectivity alterations within both MH/LH networks. Alterations in the LH network were linked with higher levels of

stress response and emotional-driven eating patterns.

Keywords: hypothalamic networks, stress reactivity, emotional nervous system, emotional eating, adolescence obesity

Introduction

Over 23% of children and adolescents in developed countries are overweight or obese[1]. In adolescents with excess weight, alterations in homeostatic regulation interact with higher emotional reactivity and stress sensitivity[2]. The hypothalamic-pituitary-adrenal axis (HPA), which regulates energy balance by increasing the motivation to eat in response to a depletion of energy stores[3], is also critically involved in stress response[4]. An important manifestation of the impact of stress on eating behavior is emotional eating, which is carried out to cope with negative affect. Stress can indeed increase food intake, specifically, a consumption of high calorie foods[5]. Adolescents with excess weight are particularly sensitive to stress[6], and common stressors of adolescence that are more prevalent in those with excess weight (i.e., peer bullying, social exclusion) can sensitize emotional reactivity and promote overeating and obesity.

The hypothalamus constitutes a major integration area for studying stress and food intake due to its central role in the HPA axis[7]. Early studies of the hypothalamus involved the lateral hypothalamus (LH) in increasing feeding[8] and the medial hypothalamus (MH) in the inhibition of eating behaviors[9]. This knowledge is mainly derived from lesioning and excitatory preclinical experiments[8,10], and the study of the influence that hunger and satiety-related peripheral signals (i.e., leptin, insulin, glucose) have on these hypothalamic nuclei[11]. However, contemporary research on the neural substrates of

feeding has highlighted the importance of hypothalamic connections with other neural regions that code the reward and affective properties of food (i.e., striatum, amygdala, hippocampus) and integrate internal and external sensory stimuli (i.e., posterior insula and somatosensory cortices)[12]. Affective and visceral information converge in the anterior cingulate cortex, which is involved in goal-directed attention and action selection[13]. In this line, a recent study[14] showed a differential whole-brain map of functional connections for the LH and MH nuclei in adults with normal weight. Interestingly, this previous research and a recent study from our group[15] have shown that adults with obesity, versus lean adults, have differences in the functional connectivity of the MH and LH circuits. However, it is unknown if these findings are also present in adolescents with excess weight.

An influential theory has proposed that the "emotional nervous system", that overlaps with the neural networks involved in feeding behavior, is importantly implicated in the control of feeding under stress and other emotional threats[16]. This network comprises the hypothalamus, the midbrain, the striatum, the amygdala, and the insula[16]. According to this theory, stressful situations would increase the functioning of the amygdala-hippocampal complex -where emotions and "food-memories" arise-, and the mesostriatal network -involved in motivational and rewarding needs-, therefore overriding areas of homeostatic food intake and mindful eating. Existing research suggests that the key regions of this "emotional nervous system" are altered in obesity, whereby sensitization in this hypothalamic-mesostriatal-limbic system can enhance stress, the motivation to eat, and emotional eating behaviors[16]. The LH has been suggested to play a predominant role in the regulation of both feeding and stress, being

particularly relevant in the preference for palatable food-cues[17]. Moreover, stressed humans show elevated ghrelin hormone levels[18], and the LH exclusively expresses the neuropeptide orexin, whose transmission to mesostriatal-limbic system has been associated with the enhancement of food reward during stress[19].

This study aims to compare whole-brain LH and MH resting-state functional connectivity in adolescents with excess weight versus normal weight. A second aim is to examine the relationship between these hypothalamic circuits and stress response measured as the cortisol response to a stress challenge. A third aim is to establish if the hypothalamic circuits related to stress are associated with emotional eating. We hypothesized that EW compared to NW groups will show higher functional connectivity between the LH/MH and regions of the limbic and reward systems, and lower connectivity between the LH/MH and areas related to the inhibition of the signals of hunger or to consciousness of the internal millieu[14,15,16]. In addition, we hypothesized that a higher functional connectivity [mainly in the excitatory LH center] with the key regions within the emotional network system (i.e., nucleus accumbens, amygdala and midbrain)[16,20] will be associated with both an increased stress response and emotional eating in the EW-group[16].

Method

Participants

Fifty-six adolescents with excess weight (EW) and 52 with normal weight (NW) were recruited via local press and social media. The main inclusion criteria were to be between 10 and 19 years old, according to the World Health Organization (WHO)

definition of adolescence[21], and to have an age and sex specific Body Mass Index (BMI) percentile between 5th and 85th for NW and at or above 85th for EW[22] (excluding participants who are underweight or those with morbid obesity). Exclusion criteria were: (i) self-reported history of traumatic brain injury, metabolic or systemic diseases impacting the central nervous system, (ii) clinical disorders (measured with Millon Adolescent Clinical Inventory) or any eating disorder (assessed by the Eating Disorder Inventory) and (iii) self-reported use of any medication. The Human Research Ethics Committee of the University of Granada approved the study, and all participants provided an informed consent.

Adolescents completed two sessions separated by a week. In the first session, participants underwent a functional Magnetic Resonance Imaging (fMRI) scan. Four subjects (3.7% of the initial sample) were excluded from the imaging analysis because of motion during the imaging scan (see details below in the functional connectivity analyses section). In the second session, a subset of 54 participants (50% of the initial sample; 32 NW and 22 EW) performed a virtual reality version of the Trier Social Stress task (TSST), where cortisol levels were assessed, and they completed the Dutch Eating Behavior Questionnaire (DEBQ) to measure emotional-eating behavior.

Measures

Imaging data acquisition: All participants were scanned at the same time of the day, between 4 and 6 p.m., after the main meal of the day (between 2 and 3 p.m). They performed a 6-min resting-state scan and were instructed to lie still with their eyes closed. We used a 3.0 Tesla clinical MRI scanner, equipped with an eight-channel phased-array

head coil (Intera Achieva Philips Medical Systems, Eindhoven, The Netherlands). A T2*-weighted echo-planar imaging (EPI) was obtained (repetition time (TR)=2000ms, echo time (TE)=35ms, field of view (FOV)=230 x 230mm, 96x96 pixel matrix; flip angle=90º, 21 4-mm axial slices, 1-mm gap, 180 whole-brain volumes). The sequence included four initial dummy volumes to allow the magnetization to reach equilibrium. We also acquired a high-resolution T1-weighted anatomical image for each subject with 160 slices (TR= 8.3 ms; TE= 3.8 ms; flip angle = 8°; FOV= 240 x 240 mm^2; in-plane resolution= 0.94x0.94x1; slice thickness= 1 mm) to discard gross radiological alterations and for preprocessing purposes.

Trier Stress Social Task (TSST): The virtual reality TSST was used to induce stress in the participants. This task asked participants to deliver a 5-minutes speech and to perform an arithmetic task in front of an evaluating committee which was presented as a virtual audience on a 3D monitor (details in Supplement 1, available online).

Cortisol measurements: Salivary cortisol levels were collected four times: before the onset of the TSST (T1), immediately after the TSST (T2) and T3 and T4 were measured 10 and 20 minutes after TSST termination. In this study, we used the area under the curve with respect to the ground (CortisolAUCg). This variable was calculated with these four cortisol measures above[23]. Greater values reflect greater cortisol levels (details on the cortisol samples analyses in Supplement 1, available online).

Eating Behavior: The Dutch eating behavior 33-items questionnaire (DEBQ)[24] was used to measure three subscales: external (10 items), restrained (10 items) and emotional (13

items) eating behaviors. In alignment with our theoretical assumptions[16], we used emotional eating. Higher scores represent greater emotional eating.

Subjective measures of hunger: We collected the ratings of hunger of those participants who underwent the TSST task both before this stress-inducing task (pre-TSST), and before the imaging session at the day of the scan (pre-scan). This was done by using a visual analogue scale (VAS) ranging from 0= 'not at all hungry' to 10 = 'very hungry'. The pre-TSST and the pre-scan ratings of hunger differed between-groups (see Table 1).

All behavioral data followed normal distribution as assessed with Kolmogorov-Smirnov tests (all $p>0.05$).

Analyses

Preprocessing and Analyses of Imaging data

The functional imaging data were processed and analyzed using MATLAB version R2008b (The MathWorks Inc, Natick, Mass) and Statistical Parametric Software (SPM12; The Welcome Department of Imaging Neuroscience, London). Preprocessing steps involved motion correction, spatial normalization and smoothing using a Gaussian filter (FWHM 6 mm). The realigned functional sequences were coregistered to each participant's anatomical scan, which had been previously coregistered and normalized to the SPM-T1 template. Normalization parameters were then applied to the coregistered functional images, which were then resliced to a 2mm isotropic resolution in Montreal Neurological Institute (MNI) space. All images were inspected for potential acquisition

and normalization artifacts. Additionally, we compared both study groups for potential differences in movement and found no significant differences [Mean Total (MT); (Standard Deviation; SD), NW= 0.038(0.026), EW= 0.042(0.029), p = 0.326; MT Translation (SD), NW= 0.042(0.034), EW= 0.046(0.030), p=0.499; MT Rotation (SD), NW= 0.033(0.023), EW= 0.039(0.031), p=0.233].

Hypothalamic seed-based functional connectivity analyses

Medial and lateral hypothalamic subregions were distinguished per hemisphere. Following prior work[14,25], respective seeds of interest were placed in the lateral (LH) (x= ±6, y=-10, z=-10) and the medial hypothalamus (MH) (x= ±4, y=-2, z=-12) using 2-mm-radius spheres. As in these previous studies, the MH included the arcuate nucleus, ventromedial and parts of the dorsomedial hypothalamus. The central voxel of the LH seed was in the most posterior part of this region to minimize overlap with the MH and obtain maximally specific functional connectivity maps. Importantly, these seeds were spatially separated by more than 6mm (>1 FWHM).

First-level t-test maps were estimated for each of LH and MH seeds by including its mean activity time-courses (extracted using marsbar toolbox[26]) together with nuisance signals as predictors of interest and no interest in whole-brain SPM12 linear regression analyses. Nuisance signals included six head-motion parameters (3 translations and 3 rotations), the time-courses representing mean signal fluctuations in white matter, cerebrospinal fluid, and the entire brain. Furthermore, to correct for subtle in-scanner movements from volume-to-volume, we identified the outliers scans present in the realigned functional imaging data using the CONN toolbox[27]. We excluded 1 adolescent

with NW and 3 with EW that had <4 minutes of data[28]. For the remaining sample, there were no significant differences in the percentage of outlier scans (mean 6.42% for NW, mean 5.39% for EW, t=0.643, p=0.522). For each participant, the actual removal of outliers scans[29] was done by entering the subject-specific variables identifying the outliers scans (i.e., one regressor per outlier) in the first-level models as covariates of no interest. These outlier scans were removed from these and the subsequent analyses. Contrast images were generated for each subject by estimating the regression coefficient between all brain voxels and each seed's time series. Then they were included in separate second-level two-sample models to assess for between-group effects together with sex, age, and a variable containing the subject-specific number of outlier volumes, to control for the subject loss of temporal degrees of freedom.

Stress response analyses

Behavioral: Repeated measures analyses were conducted in SPSS to assess Group x Time interaction on cortisol levels. To do that, in the analysis we entered the four samples across time of cortisol (T1 to T4) as different measures of the factors and group as within-group effect.

Neuroimaging: To test brain group-interactions in the association with stress response, the first-level contrast images representing the bilateral connectivity for each of the MH and LH seeds were entered in separate second-level two-sample models using the transformed into univariate variable "CortisolAUCg" (after regressing out the pre-TSST ratings of hunger) as a covariate of interest in SPM. Sex, age, the variable containing the subject-specific number of outlier volumes, and the pre-scan rating of hunger were also

included in the model as covariates of no interest.

Imaging thresholding criteria

Minimum threshold extents for the imaging analyses were calculated for all statistical comparisons by 1000 Monte Carlo simulations using the cluster-extent based AlphaSim thresholding approach[30] as implemented in the SPM RESTplusV1.2 toolbox. For the within-group LH and MH functional connectivity maps, input parameters included an individual voxel threshold probability of 0.001, a cluster connection radius of 5 mm, and the respective actual smoothness of imaging data after model estimation, incorporating a whole-brain image mask volume (224406 voxels). The minimum cluster size extent (CS) was determined to be 1016 mm^3 for LH seed (127 voxels), and 976 mm^3 for MH seed (122 voxels). For the between-group effects in the functional connectivity of the LH and MH seeds, the required cluster extent was calculated using the same input parameters specified above, but entering as masks the joined positive and negative within-group maps of the functional connectivity of the adolescents with normal and excess weight for each of the seeds (38058 voxels for the LH seed, and 16001 voxels for the MH seed). The minimum cluster size was 456 mm^3 (57 voxels) for LH seed, and 288 mm^3 (36 voxels) for MH seed.

In the analyses testing for group-interactions in the association with stress response, we applied masks focused on our target subcortical regions: the nucleus accumbens, the amygdala, and the midbrain[16,31] (see Figure S1, available online). In all cases, statistical significance was set at $p<0.05$, Family-Wise Error (FWE) corrected for multiple comparisons across all in-mask voxels (i.e., using small-volume correction procedures).

Associations with emotional eating behavior

A two-tailed Pearson correlation analysis in SPSS was conducted to test for associations between the stress associated hypothalamic networks (LH/MH seeds) and emotional eating behavior. For that, we extracted the signal from the peak coordinate of the brain regions showing significant group-interactions in the association between hypothalamic networks and stress response. These analyses were carried out separately, due to our interest in the association within each of the participants' groups and controlled by the hunger pre-TSST. Correlations were considered significant at a threshold of $p<0.05$.

Results

Stress response and association with behavioral eating scores

Repeated measures analysis showed a significant change in the mean stress response along the TSST task [$F_{(3, 153)}=11.04$; $p<0.001$], as well as a significant Group x $Time_{(T1-T4)}$ interaction on cortisol levels [$F_{(3, 153)}=7.91$; $p<0.001$], with greater stress response in EW-group.

Conversely, the variable CortisolAUCg, used for the fMRI analyses, did not differ significantly between groups ($p=0.066$; Table 1), although the *d* of Cohen showed a medium effect size ($d=0.53$). The study groups showed similar emotional eating scores ($p>0.05$; Table 1).

Lateral and Medial Hypothalamic functional connectivity

The LH maps showed higher positive connectivity with and lateral frontal cortices

(that were negatively connected with the MH seed), and the striatum. The MH maps showed higher positive connectivity with sensorimotor cortices and with a cluster comprising the nucleus accumbens and the bed nucleus of the stria terminalis at the subcortical level (Figure S2, available online). Further details about the specific brain regions included in the MH and LH functional connectivity maps in each of the study groups are reported in Table S1, available online.

Between-group differences

Lateral Hypothalamus (LH): EW-group, compared to NW-group, showed higher functional connectivity between the LH seed and the lateral orbitofrontal cortex, the ventral striatum, the anterior insula extending to the operculum, and the hippocampal gyrus, and lower connectivity with the cerebellum, and posterior cortices (i.e., the precuneus and the occipital cortex; Table 2, Figure 1).

Medial Hypothalamus (MH): EW-group, compared to NW-group, showed higher functional connectivity between the MH seed and the middle temporal gyrus, and lower connectivity with the middle frontal gyrus, pre- and postcentral gyri (Table 2, Figure 1).

Associations between hypothalamic functional connectivity and stress response

The within-group positive and negative maps of hypothalamic functional connectivity related to stress response, showed a significant positive association with the connectivity in the LH-amygdala and the LH-midbrain in the EW-group, only (see Table S2, available online). MH results did not surpass the threshold for significance neither in EW-group, nor in the NW-group. Therefore, group interactions were explored only for

the LH seed.

Group interactions of the lateral hypothalamic networks with stress response were found for the connectivity of the LH-right accumbens and the LH-midbrain. These survived corrections for multiple comparisons, which considered the number of masks used in small-volume correction procedures ($P_{FWE\text{-}SVC}$<0.05, 0.05/3 masks= 0.017; Table 3, Figure 2).

Associations of emotional eating behavior

After extracting the signal of the LH networks related to stress response, EW-group showed a positive association between the functional connectivity in the LH-midbrain network and emotional eating scores (r=0.440; p= 0.040; Figure 3). No significant associations with the LH-nucleus accumbens network were observed in this group, and no significant associations emerged in the NW-group.

Discussion

Adolescents with excess weight (EW) had higher functional connectivity between LH and orbitofrontal cortex, ventral striatum, and anterior insula, and between MH and middle temporal gyrus. Furthermore, EW-group also had lower functional connectivity between LH and cerebellum and posterior cortical areas (precuneus, occipital), and between MH and middle and precentral frontal gyri, and postcentral gyrus. Higher connectivity in LH-nucleus accumbens and LH-midbrain networks was positively associated with the stress response, with the connectivity in LH-midbrain network also showing a positive association with emotional eating in EW-group.

The differences between EW-group and adolescents with normal weight (NW) on LH connectivity fit with our hypothesis and thus, with the existing literature, which implicate the LH, not only in homeostatic feeding behavior, but also in reward and motivation[16]. The LH, the ventral striatum, the anterior insula, and the OFC are highly connected and commonly implicated in the processing of the rewarding, motivational and hedonic properties of food, whereas the hippocampus codes memory for foods[12,32,33]. Overall, higher connectivity in these networks might contribute to increased eating beyond homeostatic needs in EW. Additionally, EW-group also showed higher connectivity between the MH, which contributes to inhibit eating behaviors[9], and the middle temporal gyrus, which has been reported to be positively associated with the cognitive restrain on eating, and BMI[34]. Congruently, MH-middle temporal cortex network may underlie restrained eating behaviors in EW[35]. However, this study cannot deduce whether this brain mechanism would positively or negatively affect long-term weight-loss outcomes.

The finding of lower functional connectivity in the LH-cerebellum in EW-group is congruent with altered hypothalamic resting-state connectivity in adults with excess weight[14], and may indicate alterations in the integration of somatic and visceral information[36]. Also consistent with our previous study[14] and the literature[33] in adults with excess weight under fasting and satiation, EW-group also showed lower functional connectivity between the MH and the middle frontal gyrus and the precentral frontal gyri. These frontal areas are implicated in cognitive control, and particularly in the inhibition of hypothalamic input to induce internal signals of satiety, and promote the termination of a meal[37]. However, the lower connectivity between the MH and the postcentral gyrus in

the EW-group, does not fit with the increased connectivity found in this same brain network in adults with excess weight[14]. Taste processing and the representations of laryngeal and supralaryngeal movements are located in the postcentral gyrus[38], and hyperactivation in this cortical area has been widely reported in research on food-cues in children and adolescents with excess weight[39–42]. This may lead to an increased resting-state functional connectivity at adult ages if elevated BMI levels are maintained [43,44]. This assumption should be tested by future longitudinal studies.

The specific association between the LH network and stress response suggests that adolescents with EW who are more sensitive to stressful situations, demonstrate more mesolimbic connectivity. These mesolimbic network is part of the reward system and feedback information about the rewarding nature of environmental stimuli. Indeed, the mesolimbic areas, through their dopaminergic connections with the hypothalamus, produce a paradoxical effect of greater motivation to reward under stress[45]. Also, due to their connections with the amygdala, regulates the hedonic impact of emotionally salient stimuli (e.g., foods)[31,46]. An increased cortisol release, has been strongly suggested to impair the responsivity of this dopaminergic hubs, enhancing the food-associated drives and motivation[47,48].

This previous result is complemented by the association found between the stress-induced higher functional connectivity in the LH-midbrain network and the greater presence of emotional eating behaviors in EW[16]. This finding is interesting as several hormones and peptides have been highlighted as candidates for stress-induced palatable feeding within this specific brain network[49,50]. For instance, stress hormones may act on the midbrain dopamine segregation indirectly -by altering glucose metabolism-, and

directly -by enhancing the glutamatergic drive to dopaminergic neurons[51,52]-. Future studies are needed to formally evaluate the specific underlying neurobiological mechanism.

These results should be interpreted in the context of some limitations. The cross-sectional design of the present study prevents us from determining if the hypothalamic network´s alterations cause or are a consequence of overeating or, eventually, obesity. An fMRI version of the TSST may help to determine the direct impact of stress in hypothalamic networks. Further studies should define the potential implications of ghrelin and leptin peptides in the hypothalamic function, as well as direct measures of homeostatic status (i.e., the body's nutritional needs) versus perception of hunger (as assessed in this study). The self-reported measure of hunger was only measured in the subset of participants who completed the TSST session. Furthermore, while early signs of psychopathological disorders were identified in adolescents using the MACI, this instrument does not allow for a clinical diagnosis in our study sample. Also, although the use of global signal measurements better corrects for physiological processes of no-interest (i.e., cardiac and respiratory fluctuations)[52], providing a more specific pattern of functional connectivity[53], findings should be interpreted with caution and always together with those from other studies due to the ongoing debate about its use[54]. Also, our results characterizing the different functional connectivity within LH/MH did not surpass the FWE correction and should also be interpreted with prudence. Nevertheless, this is the first study to investigate hypothalamic connectivity network in adolescents with NW and EW, and the first to explore associations with stress and emotional eating. The several psychophysiological measures (i.e., resting-state brain connectivity, virtual reality

stressful task with cortisol measures and psychological questionnaires) make up a comprehensive set of data.

In conclusion, functional connectivity alterations within the hypothalamic networks are present at early ages during adolescence in EW. These findings complement previous studies with samples of adults with excess weight[14,20]. Our study supports the hypothesis of functional connectivity disturbances between the hypothalamus and the "emotional nervous system" in EW[16], specifically showing that an increased connectivity in the LH-midbrain network is associated with a greater stress response to stressful challenges, and non-homeostatic eating under emotional states in agreement with the comfort food model[55].

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Tables

Table 1. Demographics and Clinical Characteristics of the Study Groups

Whole Sample	**Normal weight (N=51)** Mean (SD)	**Excess weight (N=53)** Mean (SD)	Test statistics [a]	*p*
Age	15.29 (1.75)	14.64 (1.78)	1.888	0.062
BMI percentile	52.35 (24.35)	93.98 (3.98)	-12.053	0.000
Fat (kg)	13.13 (6.96)	32.17 (8.46)	-12.550	0.000
Sex (females)	31 (60.8%)	37 (69.8%)	0.936	0.333
TSST Sub-sample	**(n= 32)**	**(n= 22)**		
Age	15.75 (1.70)	15.27 (2.03)	-0.906	0.370
BMI percentile	51.28 (22.12)	95.09 (3.02)	-11.054	0.000
Fat (kg)	10.40 (3.98)	28.17 (8.11)	9.515	0.000
Sex (females)	19 (59.4%)	14 (63.6%)	0.100	0.752
CortisolAUCg	263.14 (117.7)	326.86 (122.7)	1.906	0.066
Emotional Eating [b]	24.03 (8.95)	22.23 (8.97)	-0.727	0.471
Hunger pre Scan	2.71 (2.06)	1.21 (1.57)	-3.148	0.003
Hunger pre TSST	1.82 (1.97)	0.92 (1.18)	-2.088	0.042

Note: BMI = Body Mass Index; CortisolAUCg, = Area under the curve (ground); TSST = Trier Stress Social Task.

[a]Independent samples t-tests were used to asses for between-groups differences in all cases, except for sex where chi-square tests were employed.

[b]Assessed by the Dutch Eating Behavior Questionnaire (DEBQ).

Table 2. Between Group Differences in the Functional Connectivity of the Medial (MH) and Lateral (LH) Hypothalamic Seeds

Seed	Brain region	R/L	Coordinates	t	CS	Direction
LH						
	Orbitofrontal Cortex	L	-22, 32, -16	3.5	230[a]	EW>NW
	Anterior Insula	R	44, 18, 4	3.7	104[a]	EW>NW
		L	-40, 18, -12	4.5	243[a]	EW>NW
	Ventral Striatum	R	10, 10, -2	4.5	448[a]	EW>NW
		L	-6, 10, -10	4.3	230[a]	EW>NW
	Hippocampal Gyrus	L	-32, -16, -32	4.4	78[a]	EW>NW
	Precuneus	R	2, -58, 42	3.9	58	EW<NW
	Occipital Cortex	R	6, -80, -10	4.5	569[a]	EW<NW
	Cerebellum (IV)	R	20, -72, -24	4.3	94	EW<NW
MH						
	Middle Temporal Gyrus	L	-54, -24, -6	4.1	49	EW>NW
	Middle Frontal Gyrus	R	40, 36, 30	3.9	40	EW<NW
	Precentral Gyrus	L	-54, 2, 32	4.2	39	EW<NW
	Postcentral Gyrus	L	-58, -24, 22	3.9	68	EW<NW

Note: Coordinates (x, y, z) are given in Montreal Neurological Institute (MNI) Atlas space. All results surpassed P<0.001 and a cluster size (CS) of 456 mm^3 (57 voxels) for the LH, and 288 mm^3 (36 voxels) for the MH, inside the mask of within-group effects. EW = Excess weight; NW = Normal weight. [a] same cluster.

Table 3. Group-Interactions Between Stress Response and the Functional Connectivity of the Lateral Hypothalamus Seed (LH)

LH- Brain region	R/L	Coordinates	t	CS	$P_{FWE-SVC}$
Nucleus Accumbens	R	8, 10, -6	3.4	11	0.010
Midbrain	R	8, -28, -4	3.3	12	0.010

Note: Coordinates (x, y, z) are given in Montreal Neurological Institute (MNI) Atlas space. These results surpassed a $P_{FWE} < 0.05$ following small-volume correction procedures and after Bonferroni ($P_{FWE-SVC} < 0.05$, 0.05/3 masks= 0.017)

Figure Legends

Figure 1. Between-Group Differences in the Functional Connectivity of the Lateral and Medial Hypothalamus Seeds

Note: The regions in red indicate higher connectivity in excess versus healthy weight participants, whereas those in blue show lower connectivity. The right hemisphere corresponds to the right side of axial and coronal views.

Figure 2. Brain Regions Displaying a Significant Between-Group Interaction in the Relationship Between the Lateral Hypothalamus (LH) Functional Connectivity and Stress Response

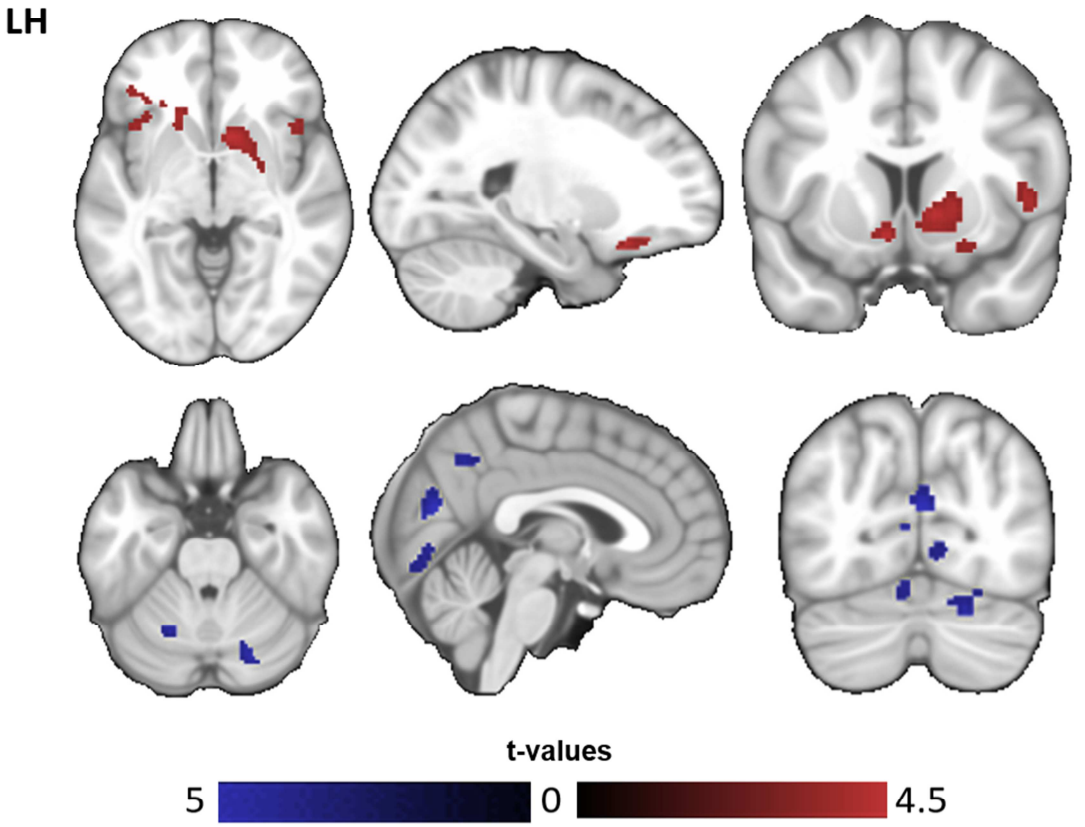
Note: The right hemisphere corresponds to the right side of axial and coronal views. Scatter plots represent the within-group correlations between the stress response (x-axis= CortisolAUCg) and the functional connectivity of the lateral hypothalamus networks in adolescents with excess weight. Positive values in CortisolAUCg indicate higher stress response, whereas negative values indicate lower stress response.

[a]corresponds to a significant correlation.

Figure 3.

Plot Showing the Correlation Between Emotional Eating and Higher Functional Connectivity Related to Stress Response in the Lateral Hypothalamus (LH)-Midbrain Network in Adolescents With Excess Weight

Note: [a]corresponds to a significant correlation.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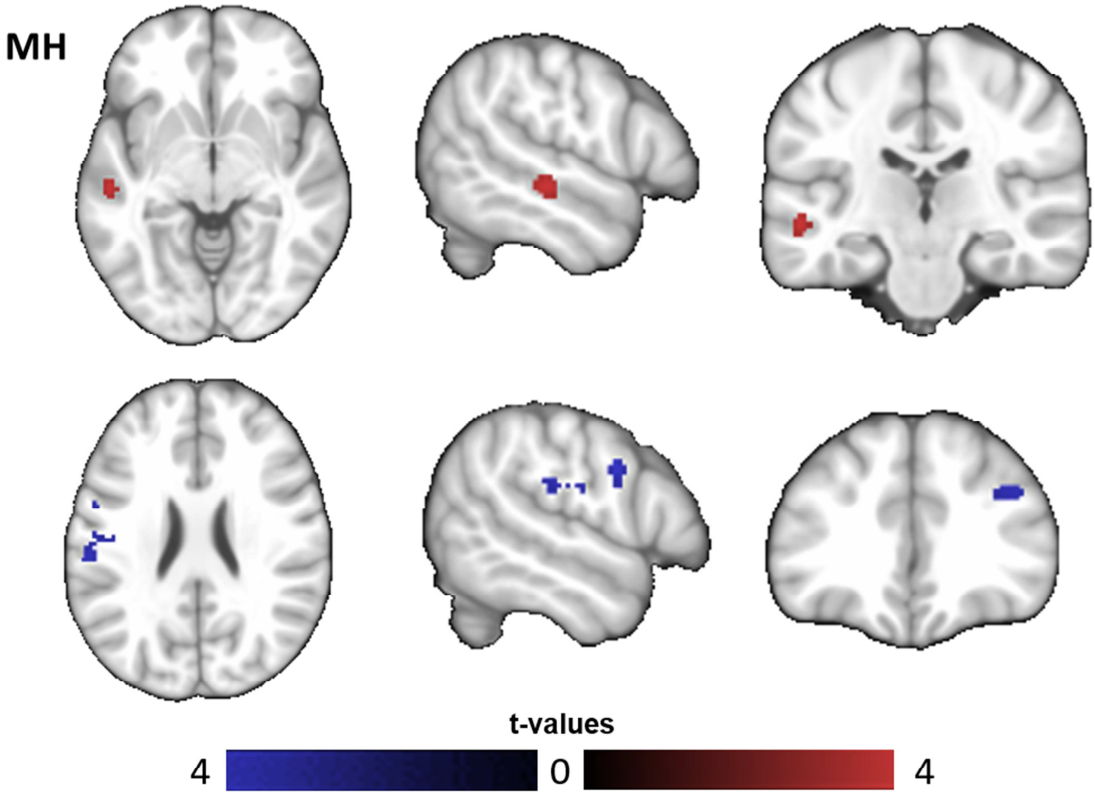
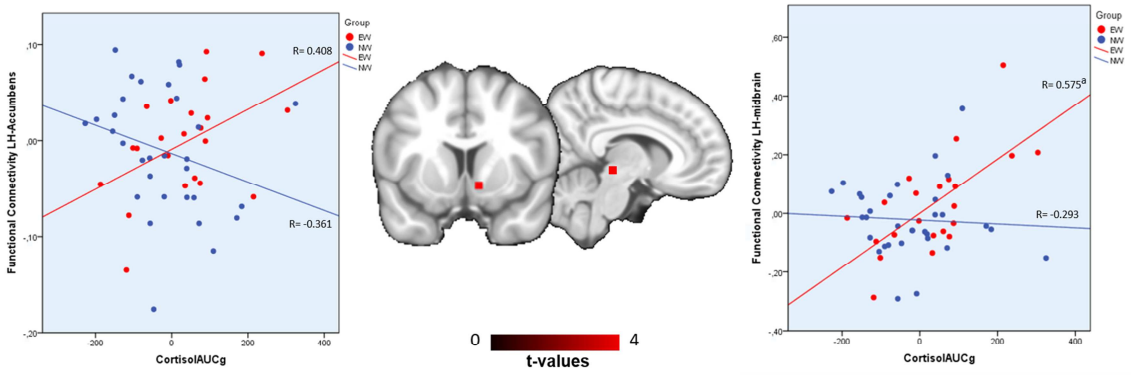
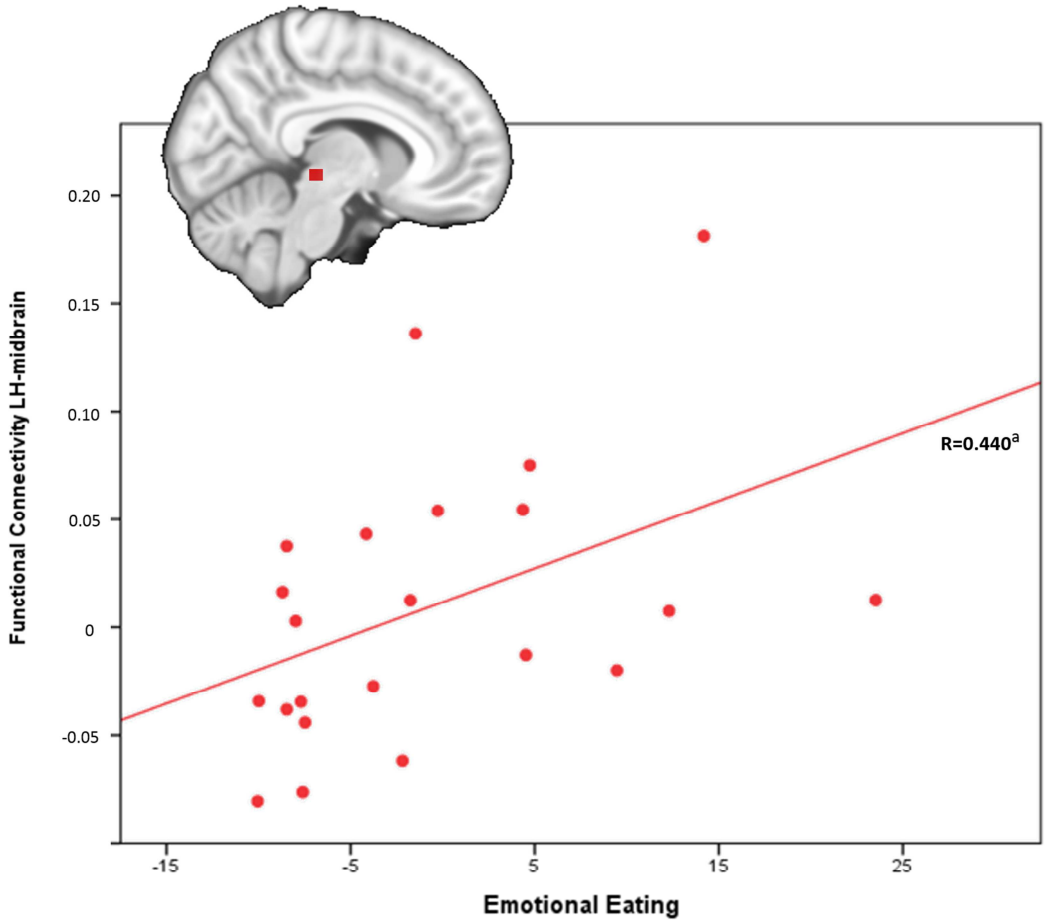
0
4.5


MH
t-values
4
0
4




Functional Connectivity LH-midbrain
0.20
0.15
0.10
0.05
0
-0.05
R=0.440[a]
-15
-5
5
15
25
Emotional Eating

Acknowledgements: This study has been funded by the Project Grant NEUROCOBE (HUM-6635) from the Andalusian Council of Innovation, Science and Industry. OCR is funded by Postdoctoral "PERIS" Contract (SLT006/17/00236) Catalan Government, 2017, Spain

Disclosure: All authors report no biomedical financial interests or potential conflicts of interest.

Table S1. Positive and Negative Functional Connectivity Within-Group Maps in Lateral Hypothalamus(LH) And Medial Hypothalamus (MH) Seeds in Adolescents With Excess (EW) and Normal (NW) Weight

Seed	Brain region	R/L	NW		EW	
			coordinates	t	coordinates	t
LH						
			Positive connectivity			
	Ventrolateral PFC	R		ns	42, 38, 0	5.9
		L		ns	-42, 36, -2	5.4
	PgACC/Medial PFC	R		ns	12, 42, 2	5.0
	Middle Frontal Gyrus	L		ns	-34, 44, 34	3.8
	Anterior Insula	R	38, 24, -6	4.5	34, 20, -10	5.5
		L	-38, 30, -10	3.5	-38, 18, -12	6.1
	Middle Insula	R		ns	40, 4, -10	4.5
		L	-42,-2,-10	3.6	-40, 4, -10	4.5
	Striatum	R	16, 0, -10	4.8	12, 8, 0	6.5
		L	-22, 6, 0	5.7	-18, 18, -4	6.0
	Hippocampus	R	22, -20, -10	4.9		ns
		L	-24, -20, -12	5.1	-26, -22, -8	5.3
	Midbrain	R	2, -26, -6	4.5	4, -26, -6	4.9
	Temporal Cortex	R	52, -16, -16	4.5	52, -16, -18	4.1
		L		ns	-48, -14, -18	5.0
	PCC	L	-6, -50, 24	3.9		ns
			Negative connectivity			
	Subgenual ACC	R	10, 28, -16	4.0		ns
	SMA	L	-10, -18, 48	4.8	-4, -26, 62	3.3
	Precentral Gyrus	R	44, -12, 56	4.5		ns
		L	-44, -22, 48	4.8		ns
	Postcentral Gyrus	R	20, -32, 58	4.6	36, -36, 56	4.3
		L	-34, -36, 56	3.5	-28, -46, 50	4.5
	Parieto-occipital Cortex	R		ns	10, -90, 38	4.0
		L	-2, -82, 32	5.1	-8, -88, 46	4.1
	Precuneus	L	-14, -44, 52	4.1	0, -56, 44	4.3
	Posterior Parietal Cortex	R		ns	40, -74, 46	3.6
		L		ns	-12, -64, -14	4.1
	Occipital Cortex	R	46, -70, 6	6.1	8, -78, -10	5.9
	Cerebellum	L	-20, -76, -22	4.5	-4, -60, -16	4.1

MH		Positive connectivity				
	ACC	R	4, 28, -4	4.4	6, 36, 6	4.6
	Anterior Insula	R	48, 2, -6		46, -8, -2	4.2
		L	-44, 4, -6	4.5		ns
	Accumbens-BNST	L	-6, 2, -4	6.3	-2, 0, -14	7.0
	Midbrain	R	4, -18, -14	5.3	0, -26, -16	4.2
	Hippocampus	R	24, -30, -14	5.2	28, -32, -12	4.6
		L	-20, -32, -12	4.5		ns
	Temporal Cortex	R	48, 2, -6	4.4	54, 0, -6	4.6
		L		ns	-54, -24, -4	5.2
	Fusiform Gyrus	R	44, -46, -18	3.7		ns
		L	-46, -46, -16	4.0		ns
	Angular Gyrus	R	52, -74, 28	5.2		ns
		L	-48, -80, 22	4.3	-48, -70, 28	3.8
	Occipital Cortex	R		ns	10, -92, 18	4.3
	Cerebellum	R		ns	26, -60, -22	4.6
		Negative connectivity				
	Dorsal ACC	L		ns	-4, 30, 30	4.4
	Middle Frontal Gyrus	R		ns	36, 50, 26	4.2
		L	-40, 34, 28	4.4	-26, 48, 30	4.3
	Superior Frontal Gyrus	L		ns	-4, 24, 62	4.6
	Temporal pole	R		ns	42, 16, -22	4.8
		L		ns	-34, 12, -26	4.4
	Precentral Gyrus	R		ns	60, 8, 26	4.6
		L		ns	-56, 4, 24	5.5
	Somatosensory Cortex	R	56, -22, 18	4.9	54, -24, 20	4.2
		L		ns	-58, -26, 20	4.6
	Occipital Cortex	L	-10, -66, 6	5.1		ns
	Cerebellum	R	14, -68, -36	4.0		ns

Note: Coordinates (x, y, z) are given in Montreal Neurological Institute (MNI) Atlas space. All results herein surpassed a height threshold of $P<0.001$ and a cluster of 1032 mm^3 (129 voxels) for the LH, and 984 mm^3 (123 voxels) for the MH, explored inside a whole-brain mask. ACC = Anterior cingulate cortex; BNST = Bed nucleus stria terminalis; LH = Lateral Hypothalamus; MH = Medial Hypothalamus; PCC = Posterior

cingulate cortex; PFC = Prefrontal cortex; PgACC = Perigenual Anterior cingulate cortex; SMA = Supplementary Motor Area.

Table S2. Positive and Negative Associations Between the Functional Connectivity of the Lateral Hypothalamus (LH) and the Stress Reactivity During the Trier Social Stress Task (TSST) Task In Adolescents With Excess Weight (EW)

Seed	Brain Region	R/L	Coordinates	t	CS	$P_{FWE-SVC}$
LH	**Excess weight**					
	Positive					
	Amygdala	R	24, 0, -18	4.0	85	0.014
	Midbrain	R	6, -30, -4	4.4	12	0.001

Note: Coordinates (x, y, z) are given in Montreal Neurological Institute (MNI) Atlas space. These results surpassed a PFWE< 0.05 following small-volume correction procedures and after multiple comparisons (PFWE-SVC<0.05, 0.05/3 masks= 0.017). LH = Lateral Hypothalamus.

Supplement 1

Cortisol measurements

For the salivary sampling, we used Salivette Cortisol (Sarstedt, Numbrecht, Germany), consisting of a small piece of cotton, which participants were told to chew during 60 seconds and two small tubes, where cotton were inserted. Saliva samples were stored at -20°C until required for assay. The samples were analyzed at the University Hospital of Granada by the electrochemiluminescence immunoassay (ECLIA) method, to be used in automatic analyzers Roche Elecsys 1010/2010 and the Elecsys MODULAR ANALYTICS E170 module.

CortisolAUCg, which is the estimation of one value comprising every measure of cortisol from zero, allows to transform a multivariate data into a univariate space, more convenient for SPM analysis. This is a standard outcome measure used in several studies as an index of stress response[1,2]. The physiological values of cortisol has been published, in a greater sample, elsewhere6 to demonstrate differences in cortisol levels between adolescents with excess weight and normal weight, and its relation to their neuropsychological performance. However, we use a different measure (CortisolAUCg), rather than the raw values of cortisol. Besides, this previous paper did not include any fMRI measure to explain the brain differences regarding stress in this population.

Trier Stress Social Task (TSST)

The speech should be about their qualities and defects. The participant must begin their speech when the curtain lifts and the virtual audience appears on the monitor. After two minutes, a change of attitude occurs in the audience, turning from an "interested audience" into a "restless audience". This will continue until the end of the speech,

regardless of the performance of the participant. Once the speech is over, the arithmetic task starts, where the participant must serially subtract the number 13 from 1022 as quickly as possible during five minutes. In case of error, they will have to start again. The virtual version of the TSST has demonstrated to be useful to explore psycho-physiological stress in several studies[3–5].

Supplemental Figures

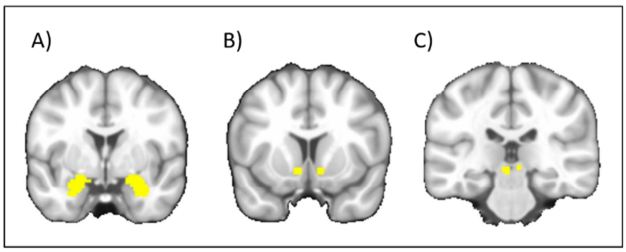


Figure S1. Bilateral Masks Used in Small-Volume Correction Procedures Represented on Anatomical Images

Note: A) Amygdala mask created using the Wake Forest University (WFU) toolbox[6] (468 voxels). B) Nucleus accumbens 3-mm spherical mask centered in Montreal Neurological Institute (MNI) coordinates, x=±9, y=9, z=-8 from Di Martino *et al*. 2008 (81 voxels). C) Midbrain 3-mm spherical mask centered in MNI coordinates, x=-4, y=-28, z=-8 for the left side and x=7, y=-28, z=-5 for the right side from Krebs *et al*., 2011[7] (81 voxels). Spherical masks were created using Marsbar toolbox[8]. The right hemisphere corresponds to the right side of coronal views.

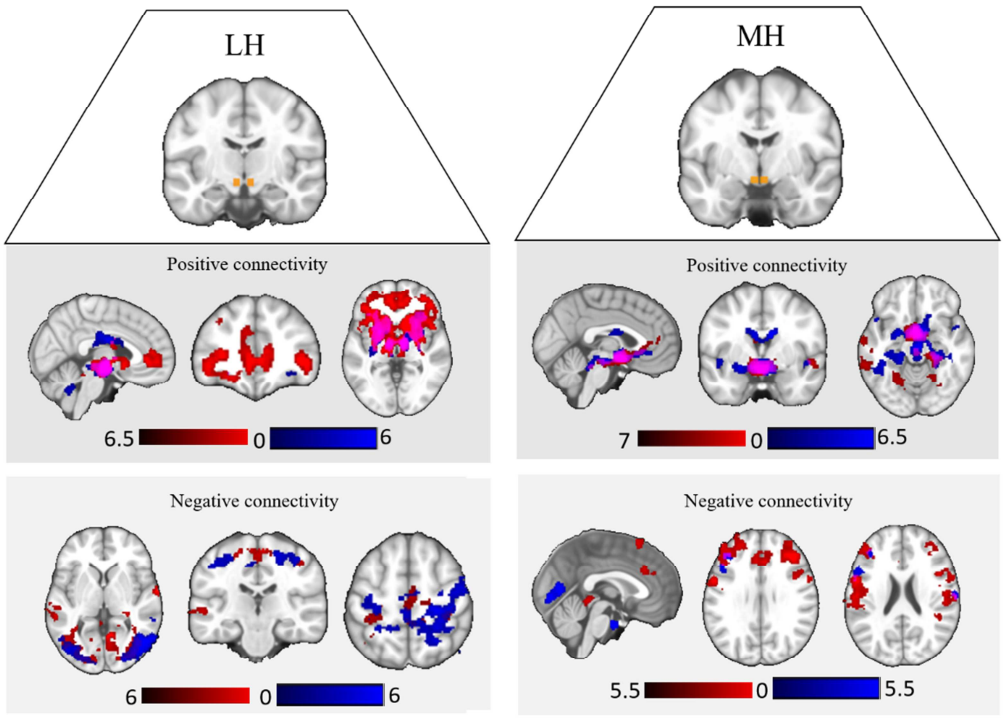


Figure S2. Positive and Negative Functional Connectivity Maps of the Lateral and Medial Hypothalamus Seeds in Adolescents With Excess (Red) and Normal Weight (Blue)

Note: Overlap between both groups is shown in violet. The right hemisphere corresponds to the right side of axial and coronal views.

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