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ARTICLE

Pediatrics

Stressing diets? Amygdala networks, cumulative cortisol, and weight loss in adolescents with excess weight

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Abstract

Objective The amygdala is importantly involved in stress and obesity, but its role on weight change and diet-related stress remains unexplored among adolescents with excess weight. We aimed to examine the functional connectivity of the Central and Basolateral amygdala nuclei (CeA and BLA) among adolescents, and to explore the longitudinal association between brain connectivity measures and diet-related cortisol and weight loss in adolescents with excess weight.

Methods We compared resting-state functional connectivity between adolescents with excess (EW, $N = 34$; Age $= 16.44 \pm 1.66$) and normal weight (NW, $N = 36$; Age $= 16.50 \pm 1.40$) using a seed-based (CeA and BLA) whole-brain approach. Then, in a subset of 30 adolescents with EW, followed-up after 3-months of dietary/lifestyle intervention, we explored for interactions between connectivity in the CeA/BLA networks and weight loss. Regression analyses were performed to explore the relationship between accumulated cortisol and weight loss, and to test the potential effect of the amygdala networks on such association.

Results In EW compared with NW, the CeA regions showed higher functional connectivity with anterior portions, and lower connectivity with posterior portions of the cingulate cortex, while the left BLA regions showed lower connectivity with the dorsal caudate and angular gyrus. In addition, higher connectivity between the left CeA-midbrain network was negatively associated with weight loss. Hair cortisol significantly predicted weight change ($p = 0.012$). However, this association was no longer significant ($p = 0.164$) when considering the CeA-midbrain network in the model as an additional predictor.

Conclusions Adolescents with EW showed functional connectivity alterations within the BLA/CeA networks. The CeA-midbrain network might constitute an important brain pathway regulating weight change.

Introduction

The prevalence of adolescent obesity has exponentially increased in the last three decades [1]. Heightened stress reactivity during adolescence is a risk factor for the development of obesity [2]. The emergence of excessive weight in this stage may lead to structural and functional neuroadaptations which may alter the balance between

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homeostatic and hedonic brain regions [3–5], thus compromising the ability to lose weight. One of the most common ways of losing weight is dieting, but this process increases psychological stress and cortisol production [6, 7]. Evidence indicates that restrictive diets induce biological stress linked to cortisol alterations [8] which, in turn, contributes to an inappropriate weight regulation, diet failure and, consequently, unsuccessful weight loss [6–8]. This vicious circle between stress-related cortisol and weight change is not entirely novel, but the role of hedonic brain regions as predictors of failed weight loss is [6, 8, 9]. Particularly, functional magnetic resonance imaging (fMRI) studies indicate that main hedonic brain hotspots (i.e., ventral striatum, ventral tegmental area, and amygdala) could be early predictive markers of weight loss outcomes after a diet intervention [9, 10] because of their crucial role on eating behavior [11, 12]. Among these areas, the amygdala stands out as a critical region in emotion processing [13, 14] and the stress response linked to a dietary process [6–8]. Under stressful conditions, the amygdala shows greater functional connectivity with several regions of the default mode network (DMN) [13, 15, 16] and with the anterior insula and pontine areas [15], regions previously involved in food reward/motivation [9, 17, 18]. Further research about the amygdala found that, under an overnight fasting, obese individuals showed a higher resting-state functional connectivity (rsFC) between the amygdala and the brain areas processing food motivation (e.g., the insula) and a weaker connectivity with the inferior frontal gyrus [19], commonly implicated in attentional control and inhibition. Similarly, another study [20] showed that participants with obesity who lost weight after a 3-week total meal replacement showed a negative functional connectivity between the dorsolateral prefrontal cortex and the amygdala during a food-cue reactivity task.

Despite the well-established role of the amygdala in stress response [3, 5], and the impact of stress on feeding and weight regulation [6–8], no studies have examined the functional connectivity of the amygdala networks in adolescents, and no study has investigated their association with longitudinal assessments of weight loss and its related cortisol accumulation in adolescents with excess weight. This is especially concerning as, compared with adults, adolescents show hyperactivity of the amygdala under negative affect and stressful situations [21, 22]. According to previous research [5], this hyperactivity could become an indicator of weight dysregulation related to non-homeostatic eating.

Two main interconnected networks have been defined within the amygdala [23]. The Basolateral Amygdala (BLA) is the sensory input nucleus and it is involved in the high-level processing of sensory and emotional input. This nucleus has predominant connections with associative cortical and striatal areas [24] and directly project to the Central Amygdala (CeA). The CeA is the major output nucleus in the amygdala. Due to its principal connections with prefrontal, autonomic and limbic areas [25], the CeA has been implicated in the behavioral and physiological responses to stress [26], and in reward motivation [27]. For these reasons, it has been suggested the importance of the CeA in the "comfort food eating" phenomenon, stimulating eating behavior during the presence of chronic stressors [28].

In the present study, we aimed to compare the functional connectivity of the CeA and BLA networks between adolescents with excess and normal weight. We hypothesized that CeA and BLA regions will show higher functional connectivity with areas related to motivational drives to eat and emotional valuation of food, but lower functional connectivity with areas related to internal awareness and eating behavior inhibition [5, 25, 27, 29, 30]. In addition, we sought to examine the longitudinal associations between CeA and BLA connectivity metrics and weight loss after a 3-month's dietary/lifestyle intervention among participants with excess weight. In line with theoretical models [31, 32], we expected associations between weight change and the amygdala connectivity with mesolimbic areas controlling food intake (i.e. hypothalamus, midbrain, nucleus accumbens). Finally, a complementary aim was to investigate whether there was a relationship between the accumulated cortisol during the 3-months' intervention and the weight loss after the intervention, and if so, to investigate whether the amygdala connectivity (especially within the CeA [12, 26–28]) has an effect in such an association [33].

Method

Participants

Seventy adolescents were recruited and classified in two groups according to their age-adjusted BMI-percentile: 36 adolescents with normal weight (NW) and 34 with excess weight (EW). In typical scenarios, the group sample sizes are enough to obtain robust between-group differences [15, 19, 20]. Participants were recruited via local newspapers, social media and ads in schools. The inclusion criteria were (i) aged 14–19 years, in alignment with the WHO definition of adolescence, including individuals aged from 10 to 19 [34], (ii) BMI-percentile between 5th and 85th for NW, and at or above 85th for EW according to their age [35]. The exclusion criteria were to have (i) comorbid medical conditions associated with obesity (e.g., diabetes, hypertension), (ii) current eating (e.g., binge eating, bulimia) or depressive disorders, and (iii) presence of structural abnormalities on the magnetic resonance imaging (MRI) or

any contraindications to MRI scanning (e.g., claustrophobia, implanted ferromagnetic objects).

Procedure

Participants completed two sessions. In the first session, they underwent a functional MRI (fMRI) scan, and their current height and weight were measured with a digital scale (Tanita DC-430U). In a second session, circa six weeks after the MRI session (mean time = 43.16 days), participants with EW received a personalized diet designed and administered by an accredited nutritionist. Both groups (EW and NW) were given standard physical activity guidelines for a healthier lifestyle.

Three follow-up sessions, where adolescents with EW were measured in their adherence to the nutritional recommendations, were done at 1, 2, and 3 months after receiving the weight loss diet. In the last session, at the end of the intervention, all the participants were weighted again to measure their weight change and a sample of hair cortisol was collected. Thirty of the initial 34 participants with EW completed all the sessions. Therefore, 34 participants with EW were included in baseline analyses (together with the 36 NW), and 30 in longitudinal analyses. Nine of these participants did not provide a sample of hair cortisol due to the following reasons: (i) they did not want to have their hair cut or (ii) did not have enough hair to collect 3 cm needed in this study (see "Hair cortisol" section in "Measures").

The Human Research Ethics Committee of the University of Granada approved the study, and all participants, and their parents if they were minors, were informed about the aim of the study and signed an informed consent.

Measures

Imaging data acquisition

All participants were scanned after the main meal of the day, approximately between 4 and 6 p.m., and performed a 6-min resting-state scan. They were instructed to lie still with eyes closed. A 3.0 Tesla clinical MRI scanner was used. It was 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 (TR = 2000 ms, TE = 35 ms, FOV = 230 × 230 mm, 96 × 96 pixel matrix; flip angle = 90°, 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.

Also, a high resolution T1-weighted anatomical image was acquired for each participant. This image, of 160 slices (TR = 8.3 ms; TE = 3.8 ms; flip angle = 8°; FOV = 240 × 240 mm; in-plane Resolution= 0.94 × 0.94 × 1; slice thickness = 1 mm) was used to discard structural alterations and for the co-registration step in the preprocessing stage.

Hair cortisol accumulation

We used the gold standard method to assess cortisol in hair, which has been validated and provides a test-retest reliability as a biomarker of chronic cortisol exposure in adults [36] and children [37] (See Supplementary material). Hair samples consisting of ~150 strands of hair were collected from the posterior vertex with a length no greater than 3 cm (assuming an average growth rate of 1 cm/month) [36]. Using a methanol extraction and ELISA, hair cortisol was detected. A 3 cm segment contains the cortisol that has been deposited over approximately the last 3 months.

Weight loss intervention

The diet intervention was composed of two parts. On one hand, every individual with excess weight was given a specific and personalized diet (considering allergies, food preferences and further information of the nutritional status given by the Krece Plus [38]). This diet consisted of a plan of meals for each day (5 per day including breakfast, morning break, lunch, afternoon snack and dinner) during the 3 months' diet. Diets were prepared for a specialized nutritionist and included all the nutrients and calories needed for every participant each day. In addition, all the participants were provided with a standard week-by-week physical activity guidelines to augment the intensity of the exercise gradually. These guidelines were based on the WHO recommendations for physical activity in adolescents.

Initial measures of the intervention

Some nutritional measures were collected the same day of the diet delivery with the KrecePlus questionnaire [38]. This test comprises 14 statements regarding healthy (+1) and unhealthy (−1) nutritional habits. The higher the score, the better nutritional status. The KrecePlus was also used to assess the baseline physical activity with questions regarding the hours that participants spend exercising or in sedentary activities (e.g., watching TV, playing videogames). These measures were used to characterize nutritional and physical activity status and create personalized diets.

Post-intervention measures

At 3-month follow-up, we re-administered the KrecePlus and, complementarily, we collected self-report information of compliance with weight loss intervention using two

questions: "Have you followed the proposed recommendations related to diet/physical activity?". These questions were answered with: "Never (0)", "Sometimes (1)", "Many times (2)", "Most of the times (3)", or "Always (4)". In addition, we collected a self-reported measure of willingness to follow with the intervention rated in a 0 (no will) to 10 (very willing) scale.

3-months weight loss

We calculated relative weight loss by subtracting follow-up weight to the initial weight, then dividing the difference by the initial weight, and finally multiplying the result by 100. This change index takes into account the initial weight in order to estimate the change. Greater positive values reflect higher weight loss.

Analyses

Preprocessing of imaging data

Functional data were preprocessed using the CONNv17 functional connectivity toolbox [39], implemented in MatlabR2017a (The MathWorks Inc., Natick, Massachusetts, USA). The preprocessing pipeline included realignment, denoising of motion artifacts and head motion (aCompCor [40]), segmentation, coregistration to each participant's anatomical scan, normalization, re-sliced to a 2 mm isotropic resolution in MNI space and smoothing using a Gaussian kernel of FWHM 6 mm. Additional steps after denoising included band-pass filtering of the BOLD time series (between 0.008 and 0.09 Hz) and linear detrending (see details in Supplementary material).

Seed definition

Seeds of interest were identified in each hemisphere using 3.5 mm radium spheres around the MNI coordinates of the basolateral (right BLA $x = +29$, $y = -3$, $z = -23$ and left BLA $x = -26$, $y = -5$, $z = -23$) and the central (right CeA; $x = 23$, $y = -5$, $z = -13$ and left CeA $x = -19$, $y = -5$, $z = -15$) amygdalae seeds using the Marsbar toolbox in MNI stereotactic space [41]. These seeds were determined following the study of the functional connectivity of the amygdala from Baur [42]. It is worth noting that all these seeds were spatially separated by more than 6 mm (>1 FWHM) to prevent overlap between signals.

Functional connectivity analyses

First-level *t*-test maps were estimated by including the time series of our seeds together with nuisance signals (WM, CSF, motion parameters) as predictors of interest and no interest in whole-brain SPM12 linear regression analyses. Separate first-level analyses were carried out for the right and the left hemisphere seeds. A high-pass filter (128-s) was used to remove low-frequency drifts. Contrast images were generated for each subject by estimating the regression coefficient between each seed's time series and all brain voxels. The first-level contrast images were then included in four separate second-level two-sample models to assess for between-group effects in each seed connectivity (R/L BLA and CeA) together with sex and age as covariates of no-interest. Besides, to test the association between the amygdala functional connectivity and weight change only in adolescents with excess weight (who followed the weight loss intervention), the first-level contrast images representing the connectivity for each BLA/CeA seeds were entered in four separate second-level multiple regression models (left/right CeA and left/right BLA) using the variable "weight change" as a covariate of interest. Sex and age were as well included in the model as covariates of no interest.

Imaging thresholding criteria

The minimum threshold extents for the functional connectivity analyses were estimated by 1000 Monte-Carlo simulations using the cluster-extent based AlphaSim thresholding approach [43], implemented in the SPM RESTplusV1.2 toolbox to control for false positive findings that can result from the multiple comparisons in voxel-based analyses. For the within-group connectivity maps, we included as input parameters an individual voxel threshold probability of 0.001, a cluster connection radius of 5 mm and the actual smoothness of imaging data after model estimation, incorporating a whole-brain image mask (240,405 voxels). The minimum cluster size for each seed was 178 voxels (1424 mm^3) for left CeA, 181 voxels (1448 mm^3) for right CeA, 187 voxels (1496 mm^3) for left BLA and finally, 192 voxels (1536 mm^3) for right BLA. For the between-group effects in the functional connectivity of the amygdalae seeds (i.e., left and right CeA and BLA) and their association with weight change in the adolescents with EW, the required cluster extent was calculated including the same parameters except for the mask, which was created by joining positive and negative within-group maps of the functional connectivity of the adolescents with NW and EW for each seed (masks: 11,486 voxels for the left CeA, 15,537 voxels for the right CeA, 37,802 voxels for the left BLA, and 47,933 voxels for the right BLA). The minimum cluster size was 42 voxels (336 mm^3) for left CeA seed, 54 voxels (432 mm^3) for right CeA seed, 89 voxels (712 mm^3) for left BLA seed, and 107 voxels (856 mm^3) for right BLA seed.

Behavioral measures and linear regression analyses

Additionally, we tested whether hair cortisol predicted weight change by performing a regression analysis with hair cortisol as a single predictor. Another regression in which we included amygdala networks connectivity as an additional predictor was performed to explore the effect of the inclusion of amygdala connectivity in the cortisol-weight change association. The regression analyses were considered significant at a threshold of $p < 0.05$. All the data were normally distributed and all the statistics were two-sided.

Results

Sample characteristics and diet-related variables

The EW and HW groups did not differ in age or sex (shown in detail in Table 1). Regarding the EW group, they showed a negative relationship between Weight Change and Hair Cortisol accumulation ($R = -0.539$; $p = 0.012$), and a positive association between Weight Change and compliance with diet ($R = 0.446$; $p = 0.013$) and physical activity ($R = 0.585$; $p = 0.001$) recommendations, the nutritional status in the follow up ($R = 0.385$; $p = 0.035$) and the willingness to follow the intervention ($R = 0.623$; $p = 0.000$). No significant associations were found between the accumulated cortisol in hair and the diet-associated variables.

CeA and BLA functional connectivity

The within-group maps of positive and negative functional connectivity showed similar results as these reported in other studies. These results are detailed in Fig. S1 and Table S1 within the Supplementary material. The between-group differences of the functional connectivity within each of the amygdala seeds are shown below:

CeA

Adolescents with EW, compared with those with HW, showed higher functional connectivity between the left CeA and perigenual anterior cingulate cortex (pgACC), extending to the medial prefrontal cortex, and lower connectivity between the right CeA and the posterior cingulate cortex (PCC) (Table 2; Fig. 1).

Table 1 Demographics and clinical characteristics of the groups.

	Normal weight ($N = 36$) Mean (SD)	Excess weight ($N = 34$) Mean (SD)	Test statistics[a]	p value
Age	16.50 (1.40)	16.44 (1.66)	0.160	0.873
BMI percentile	50.33 (19.31)	93.74 (4.27)	−13.150	0.000
Fat (%)	21.28 (8.95)	30.49 (5.68)	−5.172	0.000
Sex (females)	19 (52.8%)	19 (55.9%)	0.068	0.794
Hunger pre-Scan	18.50 (18.99)	18.91 (20.41)	−0.087	0.931
	EW weight lost ($n = 21$)	EW weight gain ($n = 9$)		
Age	16.76 (1.64)	15.89 (1.45)	−1.379	0.179
Sex (females)	10 (47.6%)	6 (66.7%)	0.918	0.338
Nutritional status baseline	5.80 (1.74)	5.89 (1.76)	0.127	0.900
P.A. status baseline	4.24 (1.79)	5.44 (2.51)	1.500	0.145
Weight change (%)[b]	−5.64 (3.07)	4.24 (2.83)	8.543	0.000
Diet compliance	2.38 (1.07)	1.44 (0.73)	−2.782	0.011
P.A. compliance	2.52 (1.03)	1.78 (0.83)	−1.914	0.066
Nutritional status follow-up	6.76 (1.51)	5.89 (2.18)	−1.275	0.213
P.A. status follow-up	5.00 (1.90)	5.44 (2.13)	0.567	0.575
Willingness	7.8 (1.54)	5.94 (1.38)	−3.228	0.005
Hair cortisol	$n = 15$, 251.13 (111.34)	$n = 6$, 395.47 (59.58)	3.833	0.001

Levene's test showed equal variances between our study groups

P.A. physical activit

[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]For a more intuitive interpretation of the results, weight lost was indicated in this table as a negative value representing the percentage of weight loss.

Table 2 Between-group differences in the functional connectivity of the central (CeA) and basolateral (BLA) amygdala seeds.

Seed	Brain region		*x*, *y*, *z*	*t*	CS	Direction
CeA						
	Right seed					
	Posterior cingulate cortex	R	10, −40, 22	4.8	312	EW < NW
	Left seed					
	Perigenual ACC extending to OFC	R	8, 46, 2	5.2	823[a]	EW > NW
	Medial prefrontal cortex	R	2, 46, 24	4.5	823[a]	EW > NW
BLA						
	Left seed					
	Dorsal caudate	R	10, 20, 6	4.7	175	EW < NW
		L	−10, 22, 4	4.4	125	EW < NW
	Angular gyrus	R	48, −70, 22	4.2	150	EW < NW

Coordinates (*x*, *y*, *z*) are given in Montreal Neurological Institute (MNI) Atlas space.

CeA central amygdala, *BLA* basolateral amygdala, *EW* excess weight, *NW* normal weight, *CS* cluster size, *ACC* anterior cingulate cortex, *OFC* orbitofrontal cortex.

[a]Indicates part of a larger cluster. All results herein surpassed a height threshold of $P < 0.001$ and a cluster of 336 and 432 mm^3 (42 and 54 voxels) for the left and right CeA seeds, respectively, and 712 and 856 mm^3 (89 and 107 voxels) for the left and right BLA seeds, respectively, explored inside the mask of within-group effects.

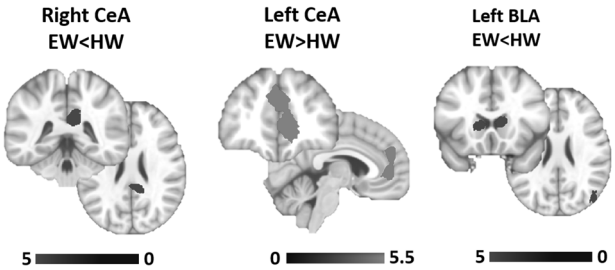

Fig. 1 Between-group differences in the functional connectivity of the different seeds of the amygdala (right/left CeA and left BLA). The regions in red indicate higher connectivity in excess versus normal weight participants, whereas those in blue show lower connectivity. The right hemisphere corresponds to the right side of axial and coronal views.

BLA

Adolescents with EW, compared with those with HW, showed lower functional connectivity between left BLA and the right angular gyrus and both dorsal caudate nuclei (Table 2; Fig. 1).

SPM correlation between the amygdala functional connectivity and weight change

In the adolescents with EW, weight loss was negatively associated with the functional connectivity between the left CeA and the midbrain (Coordinates = 14, −14, −12; $t = 5.6$; cluster size = 76; $p < 0.001$; see Fig. 2). No other association surpassed the significance threshold.

Additional regression analyses

CeA-midbrain rsFC was significantly related to weight change ($R = -0.734$; $p < 0.000$) and hair cortisol ($R = 0.466$; $p = 0.033$). In addition, hair cortisol accumulation significantly predicted weight change ($R = -0.539$; $p = 0.012$). However, this latter association was no longer significant when we included CeA-midbrain rsFC as an additional predictor in the model ($B = -0.012$; $p = 0.164$). The best fitting model included only CeA-midbrain rsFC as predictor ($B = -23.660$; Adjusted $R^2 = 0.547$; $p < 0.000$).

Discussion

In a first aim, we sought to characterize the rsFC of the amygdala in adolescents with excess weight (EW) in comparison with their normal weight (NW) counterparts. The adolescents with EW showed alterations in the functional connectivity between the central amygdala (CeA) and anterior and posterior parts of the DMN, while the basolateral amygdala (BLA) showed a lower rsFC with the bilateral dorsal caudate.

The lower rsFC in the right CeA–PCC network has been previously associated with lower interoceptive awareness about emotional states [44]. Available studies have suggested a joint altered activation of the PCC and amygdala during stress, with the alterations in the posterior DMN area being also reported in obese conditions [18, 45]. Interestingly, a study with siblings [46] has suggested this DMN alteration to be a consequence rather than a predisposing factor of obesity, and thus it may improve after significant weight loss.

On the other hand, the heightened rsFC in the left CeA–ACC/mPFC network has been more extensively examined and is one of the most replicated association of the amygdala in relation to emotional processes [13–16], also in adolescents [47]. These prefrontal regions, in coordination with the amygdala, are known to automatically react to biologically relevant environmental stimuli [48], as well as to the derived endogenous cortisol levels [13]. Indeed, the specific area of the ACC found in our study, the pgACC, regulates autonomic and affective responses by evaluating the emotional valence of complex stimuli and

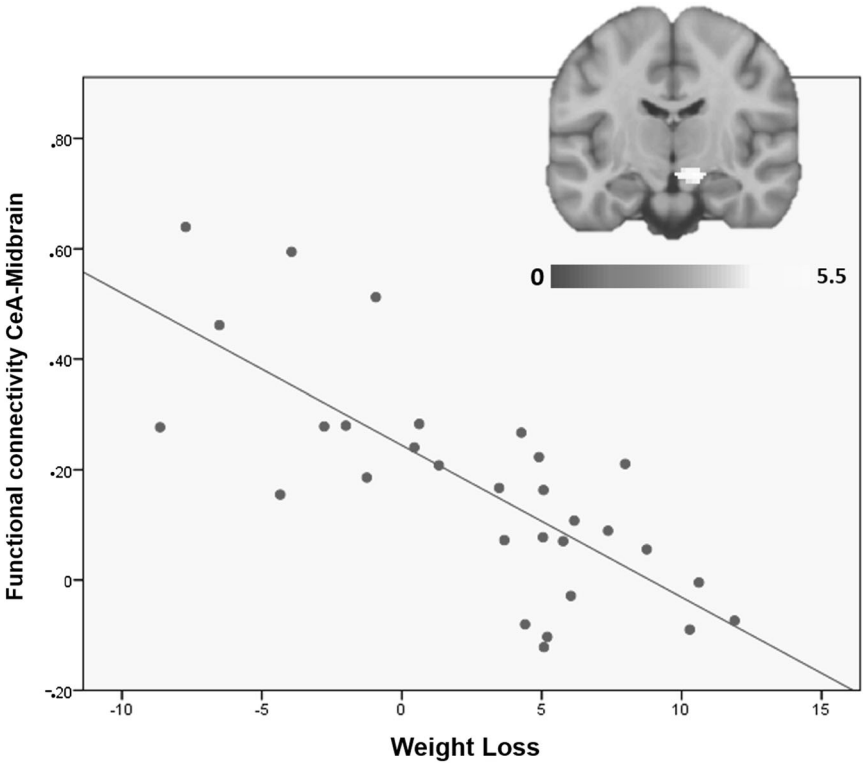

Fig. 2 Plot showing the significant negative correlation between the functional connectivity of the CeA-midbrain network (MNI coordinates *x*, *y*, *z*: 14, −14, −12) and the weight loss after a 3 months' intervention in adolescents with excess weight. The right hemisphere corresponds to the right side of axial and coronal views. Positive values in "weight loss" indicate greater weight loss, whereas negative values indicate a lower weight loss.

orchestrating the emotional response together with the amygdala [14]. A study with normal weight adolescents showed that stronger connectivity in this brain network is linked to better regulation of fear responses [47]. Therefore, and consistent with previous studies, a higher connectivity in our sample of adolescents with excess weight may be related to the altered emotion regulation processes in youth [49] due to difficulties to disengage from negative events [50] or, alternatively, to the use of maladaptive emotional regulation strategies [21], such as emotional eating [50] or food addiction-related behaviors [51]. Altogether, the altered connections between the CeA and anterior and posterior areas of the DMN may make adolescents more susceptible to affective dysregulations [47, 52], including heightened stress reactivity.

The lower rsFC we found between the left BLA and both dorsal caudate nuclei in EW contrasts from the increased functional connectivity to the sight of appetizing foods reported in this same network in adults with morbid obesity [53]. However, other studies showed caudate activity to be inversely related to BMI [54] and weight gain [55]. Our findings may be congruent with hypotheses pointing toward a reward circuitry hypofunction while resting, but hyper-responsive to food cues [54]. However, because of the lack of studies in adolescents with excess weight, longitudinal studies are needed to discard for changes in BLA connectivity from adolescence to adulthood.

As a second objective, we aimed at exploring the association between the amygdala rsFC and weight change during the 3-months intervention, and the potential role of accumulated cortisol during the intervention on the significant weight-associated amygdala rsFC, and the total weight loss in the adolescents with EW. We found a negative relationship between the accumulated cortisol and weight change. This result is congruent with previous studies showing the implication of cortisol accumulation in eating behavior [28, 32] and during weight loss efforts [6, 8]. From a biological perspective, the accumulation of cortisol leads to fat storage [56] which may impair homeostatic-driven eating behaviors [28], potentially leading to less weight loss in the dieting process [6]. Further studies also highlight the long-term effects of hair cortisol over the persistence of obesity [56]. However, this relationship was no longer significant after the inclusion of the CeA-midbrain as a variable in the regression model. This finding indicates that connectivity strength within this network, part of the hedonic brain system, may be a biomarker of the weight loss difficulties during an intervention [31]. Particularly, adolescents with excess weight with higher functional connectivity in CeA-midbrain network at the beginning of an intervention may be those with greater problems to lose weight. The significance of this brain networks on appetitive behaviors is beginning to be understood [27]. Stressful and associated arousing negative

events, such as dieting [6], enhance the mesolimbic/limbic system's function [33], where the midbrain and the CeA are located [31]. Beyond the association of the amygdala–midbrain network with the stress response, the hypothalamus-midbrain network has also been associated with stress reactivity in a recent study [4]. Importantly, these three brain areas form the theoretical "limbic triangle" proposed by Mietus-Snyder and Lustig [31]. According to this model, the hypothalamus and the midbrain mediate satiety-related homeostasis, but the amygdala circuits may override homeostasis during stressful events [31]. In addition, less weight loss, but not cortisol accumulation, was associated with lower intervention adherence and willingness to continue it. Nonetheless, based on the association between cortisol accumulation and diet success found here and in previous studies [6–8], cortisol levels during dieting may be considered in the development of successful interventions.

The present study has several limitations to be acknowledged. First, this study cannot determine if the alterations shown in the amygdala networks are a previous vulnerability or a consequence of excess weight, due to our cross-sectional design in the imaging variables. A second fMRI scan after the intervention could have provided more information about the potential brain changes related to weight loss. Another limitation of our study relays in the omission of self-reported measures of stress, which may complement the cortisol assessment. Also, to increase the internal validity, we discarded those participants with depression or binge eating disorder, and this may have limited the external validity of this study. Notwithstanding the limitations, this study is the first to explore the functional connectivity of the different regions of the amygdala in adolescents, and its association with the accumulated cortisol and weight change over a 3 months' intervention. Moreover, our study comprises hormonal, neural and behavioral measures, and contains longitudinal follow-ups in several measures along the intervention. This study highlights the importance of the brain non-homeostatic pathways for the success of a weight change intervention, and how the accumulated cortisol during the dieting process could dampen the weight loss.

We conclude that the functional connectivity strength in the CeA-midbrain network is relevant to explain the effects of stress on weight change difficulties during an intervention among adolescents with excess weight. This knowledge could be applied in further studies that aim to develop prognostic signatures of weight loss and surrogate markers of treatment response.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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