VU VRIJE UNIVERSITEIT AMSTERDAM

VU Research Portal

Obesity and Food Reward Regulation by the Brain

Doornweerd, S.

2018

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

citation for published version (APA)
Doornweerd, S. (2018). *Obesity and Food Reward Regulation by the Brain: Genetic and Environmental Factors*. [, Vrije Universiteit Amsterdam].

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:
vuresearchportal.ub@vu.nl

Download date: 11. Nov. 2024

Polygenic risk to obesity and alterations in fMRI brain reward system responses to food in females

VIII

Stieneke Doornweerd, Eco J de Geus, Dick J Veltman, Jenny van Dongen,
Madeleine L Drent, Dorret I Boomsma, Richard G IJzerman,
Submitted

ABSTRACT

Obesity has been linked to altered brain reward responsiveness to food, although the causal direction of these associations remain unclear. Body weight is highly heritable and genome wide association studies have successfully identified obesity-associated single nucleotide polymorphisms (SNPs). Effects of these variants are small, but can be aggregated in a polygenic risk score (GRS). We aimed to investigate whether altered food reward responses are explained by a GRS comprised of 77 obesity-associated SNPs or reflect a consequence of increased BMI itself. Using a four corner model, we studied 60 females (BMI range 17.4 – 43.9 kg/m^2) who were selected to have either a low or high GRS for obesity and low or high measured BMI, from a sample of ~11,000 people. Functional MRI scans were obtained during the presentation of low-calorie, high-calorie and non-food pictures, and during the anticipation and receipt of chocolate milk. Individuals with high versus low GRS had greater activation in the right orbitofrontal cortex during chocolate milk anticipation, irrespective of current BMI. In response to chocolate milk consumption, individuals with high versus low BMI had greater activation in bilateral amygdala, irrespective of GRS. No effects of GRS, BMI or their interactions were observed on activations to watching (high-calorie) food pictures. Our findings support the notion that genetic predisposition to obesity may impact on weight through increased reward responsiveness to anticipatory food cues. Increased BMI itself may lead to increased valuation of palatable food receipt which may lead to overeating and weight gain in a food-abundant environment.

INTRODUCTION

The excessive eating in obesity is suggested to result from food intake driven not by nutritional needs, but by the rewarding properties of highly palatable energy-dense foods that override homeostatic signals of the body [1]. In previous functional MRI (fMRI) studies we [2,3] and others [4-6] demonstrated that obese compared to lean individuals have increased responses to appealing food pictures and to cues that signal the delivery of palatable food [7] in brain regions mediating reward and motivation, such as the insula, striatum and orbitofrontal cortex (OFC). Results from prospective studies [8,9], demonstrating that this hyper-responsiveness to food predicts future weight gain, suggest a causal role in the development of obesity. Additionally, a lower striatal response to the actual consumption of a palatable food has been observed in obese versus lean individuals, as assessed with fMRI [10,11]. Evidence from animal experiments [12] and longitudinal studies in humans [13] suggests that this reduced striatal response to food consumption results from habitual overeating of energy dense palatable food.

Body weight is highly heritable [14] and genome wide association studies (GWAS) have successfully identified polymorphisms that contribute to common obesity [15,16]. Several findings suggest that these loci may be implicated in the regulation of food reward and appetite by the brain. Rare monogenic mutations that cause severe early-onset hyperphagia and obesity have shown to involve pathways in the brain that regulate appetite, energy balance and reward [17,18]. Furthermore, genes that are repeatedly linked to common obesity (such as *FTO* and *MC4R*) are highly expressed in the hypothalamus [15] and are associated with altered reward responsiveness to food cues [19-21].

However, the pathways by which other recently detected single nucleotide polymorphisms (SNPs) influence BMI are mostly unknown. In addition, the effects of individual SNPs on BMI are small, and studies investigating single SNPs may consequently have a limited ability to detect subtle differences in brain reward responses to food. Combining the effects of multiple genetic variants in a polygenic risk score (GRS) [22] may be more useful to study alterations in brain reward system functioning caused by genetic variation. Therefore, in the present study we used a 77 SNPs GRS to identify individuals to be at either low or high genetic risk for obesity and investigate whether this difference in genetic susceptibility to obesity is related to differences in brain reward responsiveness to food, which could act as an intermediate causal step in the development of obesity.

Further classification of this study sample into individuals with either a low or high current BMI provides the opportunity to investigate whether altered brain reward responsiveness to food reflects a cause or, rather, a consequence of increased BMI. Specifically, alterations in brain reward responsiveness to food that are related to current BMI irrespective of GRS for obesity are more likely to be secondary to high

BMI. In contrast, alterations in brain reward responsiveness to food that are related to GRS for obesity may be an etiological factor contributing to genetic susceptibility to obesity. This approach is based on the previously established 'four corners epidemiological model' [23-25] which, in a similar fashion, demonstrated that defective angiogenesis may be an etiological component in the genetic predisposition to high blood pressure, rather than being a consequence of increased blood pressure as such [23]. In fact, we improved the four corners design by using measured GRS instead of parental characteristics to define genetic predisposition, as in the original design. By doing so, we excluded the influence of confounding effects of familial environmental factors, which still explained part of the familial predisposition to the trait of interest in earlier studies.

We hypothesized that increased responsiveness to anticipatory food cues in brain regions mediating reward and motivation is a feature of the genetic predisposition to obesity, whereas a decreased striatal response to consuming a palatable food stimulus may, in contrast, result as a consequence of increased BMI.

SUBJECTS AND METHODS

SUBJECTS

Participants were recruited from the Netherlands Twin Register (NTR), comprising twins and their family members for longitudinal survey studies on health and disease [26, 27]. For the current study, participants were selected in two phases (for a flow chart, see Figure 1 in Chapter 7). In the first phase, all registered individuals were selected who had undergone DNA extraction and genotyping as part of previous genome-wide association studies (GWAS) [15, 28]. Genotyping was done as described in detail previously [27]. For each of these 11,495 individuals, a polygenic risk score (GRS) for obesity was calculated using 77 known obesity SNPs in individuals of European descent from the most recent meta-analysis of GWAS in adults [16]. The 77 loci were used that reached genome-wide significance for BMI ($P<5 \times 10^{-8}$) in the European analysis in males and females. A summed weighted GRS was calculated by summing the BMI-increasing alleles after weighting the alleles by their effect sizes [22]. Before running a second selection phase, individuals were excluded if they 1) had no weight and/or height recorded during the latest NTR biobank study [29], necessary for calculating recent BMI, 2) were part of monozygotic twins or triplets, or 3) were part of the same family as another individual in the sample. The removal of all but one member of a same family was done to create a sample of genetically unrelated individuals. To this end, spouses and parents of registered twins were first selected followed by randomly chosen siblings or members of twin pairs.

For the second selection phase the sample was then divided into four corners using cut-offs that corresponded to the lower and upper 25% of

the distribution of BMI (i.e. ≤ 22 kg/m^2 and ≥ 27 kg/m^2) and the lower and upper 20% of the distribution of GRS (i.e. ≤ 0.90 and ≥ 1.03). This resulted in four groups of individuals identified as having either low GRS/low BMI, low GRS/high BMI, high GRS/low BMI or high GRS/high BMI. Finally, because of earlier reported gender differences in responses to food cues, with females showing higher activations than men [4], we excluded all male participants from this final sample.

Thus, after confirming whether individuals were still registered as active participants in the NTR, a total of 248 letters were sent with detailed information and an invitation for participation in the study. Through telephone contact more information was provided when needed, and exclusion criteria were checked, i.e. the presence of diabetes mellitus, serious heart, liver or renal disease, malignancies, uncontrolled thyroid disease, neurological or psychiatric disease including eating disorders and depression [30], pregnancy or lactation, alcohol or drug abuse, the use of glucose-lowering drugs or psychoactive medication, history of bariatric surgery, recent weight change (>5% reported weight change in previous 3 months) and MRI contra-indications. In total 113 women were unwilling to participate because of lack of time or travel time being too long. Of the remaining women, 46 were excluded due to recent body weight change (n=15), co-morbidities or medication (n=23), pregnancy (n=4) or MRI contra-indications (n=4). Another 29 individuals could not be contacted by telephone due to loss of follow-up information. Thus, 60 women were included in this study: 16 women with low GRS/low BMI, 12 with low GRS/high BMI, 15 with high GRS/low BMI and 17 with high GRS/high BMI. The study was approved by the VU University Medical Centre ethics committee and performed in accordance with the Helsinki Declaration. All participants provided written informed consent.

MEASURES

Clinical assessments Participants arrived at the clinic between 8:00 and 10:00 AM after an overnight fast. Information on health and socio-demographics was collected using standardized oral interviews. Handedness was assessed using a validated questionnaire [31]. Weight, height and waist- and hip circumference were measured in a standardized manner, as described in detail previously [32]. BMI (kg/m^2) was calculated to reflect body fatness. Body composition was measured using bio-electrical impedance analyses. Venous blood samples were drawn for the assessment of fasting glucose and lipid spectrum.

Hunger and appetite Before the scanning session participants were asked to rate their feelings of appetite and hunger on a Likert scale ranging from 0 ('not at all') to 10 ('extremely'). Participants were asked the questions 1) How hungry are you now? 2) How full are you now? 3) How much could you eat right now? 4) How much is your desire right now to eat something sweet / savoury / fat right now?

Food stimuli ratings After the scanning session participants viewed all 84 food pictures that were presented during the fMRI scan and rated each picture on how palatable the food in the picture appeared to them at that moment on a 7-point Likert scale ranging from 1 ('not at all') to 7 ('extremely'). On a similar scale participants rated the palatability of the receipt of the chocolate milk and tasteless solution used in the fMRI paradigm.

TASK PARADIGMS

Both imaging paradigms used in this study were described in detail previously [2, 3, 10].

Food picture paradigm Blood-oxygen level dependent (BOLD) signal was measured during the presentation of 42 pictures of high-calorie food (e.g. pizza, French fries, ice cream, donuts), 42 pictures of low-calorie food (e.g. fruit salads, an apple, green vegetable salads, a cucumber) and 42 pictures of non-food items (trees, bricks, stones), all pictures being matched for colour, shape and size (see Figure 1A in Chapter 5). Using a block-design, 7 same-category pictures were presented in each block for 2.5 sec per picture, separated by a 0.5 sec blank screen. At the beginning of the scanning session participants were instructed to watch each picture attentively. To test their concentration during the task, one hour after the scan a recognition test was performed comprising 20 pictures of which participants needed to identify the10 pictures that were previously presented in the scanner.

Chocolate milk paradigm In a second fMRI task, BOLD signal was measured during the anticipation and delivery of chocolate milk (Chocomel, 86 kcal, 2.7 g fat, 11.8 g sugar per 100 ml) and a tasteless solution (see Figure 1B in Chapter 5). The tasteless solution was used as a neutral stimulus, designed to mimic the natural taste of saliva and consisted of 2.5 mM NaHCO3 and 25 mM KCL [11]. Two images were presented (an orange triangle or a blue star) that signalled the delivery of either 0.4 ml chocolate milk or tasteless solution, respectively. Images were presented for 2 sec (i.e. anticipation time) in random order, followed by 3 sec of grey screen with a fixation cross and 2 sec of stimulus delivery (i.e. receipt). Participants were instructed to keep the solution in their mouth during 6 sec until the sign 'swallow' appeared. The next trial was started after a jitter of 3-7 sec (i.e. baseline period). In 40% of the events, the cue was not followed by a stimulus delivery [7]. Tastes were delivered using programmable syringe pumps and Vygon syringes that were inserted into the participant's mouth.

IMAGING ACQUISITION AND ANALYSIS

Imaging data were acquired using a 3.0 Tesla GE Signa HDxt scanner (General Electric, Milwaukee, WI, USA). For structural imaging, T1 weighted scans were acquired using a 3D fast spoiled gradient-echo

sequence. For the functional data, a T2* weighted gradient echo-planar imaging sequence was used (repetition time/echo time = 2160/30 msec, flip angle 80°, slice thickness 3 mm, matrix size 64 x 64, 211 x 211 mm^2 field of view, voxel size 3 x 3 x 3 mm, 40 slices).

Pre-processing was done using SPM8 software (Wellcome Trust Centre for Neuroimaging, London, UK) run within Matlab R2012a (Mathworks, Inc.). The origin of each imaging volume was aligned to the anterior commissure. Functional images were realigned to the first volume to correct for head motion, and slice-time corrected to the onset of the middle slice. Further, data were co-registered to the structural scan and segmented to be spatially normalized to the standard Montreal Neurological institute (MNI) template. Finally, images were spatially smoothed using an 8 mm full-width at half maximum Gaussian kernel. The functional data were passed through a high-pass filter (cut-off 128 sec) to remove low-frequency artefacts. No data set showed within-run head movement of more than 3 mm (translation) or >2.5° (rotation).

Block-design BOLD-responses were analysed within the context of the general linear model, as implemented in SPM8. At the first level, for each participant statistical contrasts were generated for comparing brain activations to 1) watching food vs. non-food pictures, 2) watching high-calorie vs. non-food pictures, 3) anticipating chocolate milk vs. baseline, and 4) chocolate milk receipt vs. baseline. Baseline was defined as the jittered time between trials, with removal of the first 3 sec [33]. To specifically assess the effect of anticipating and receiving a palatable taste stimulus as opposed to anticipating and receiving a taste stimulus in general, contrasts were also generated for 5) anticipation of chocolate milk vs. tasteless solution and 6) receipt of chocolate milk vs. tasteless solution.

STATISTICS

Clinical data Clinical and behavioural data were analysed using IBM SPSS Statistics (version 20, IBM Corp., 2011, Armonk, NY). Results are expressed as mean ± sd. Differences between the groups were tested using a 2 by 2 factorial ANOVA. *P*-values were calculated and reported for separate effects of GRS and BMI, and for the interaction between the two factors [24]. Chi-square test was used for the analyses of categorical variables. Palatability ratings of high-calorie and low-calorie food pictures were analysed using a mixed between-group (2 by 2 factors) within-group (2 food categories) ANOVA [5]. A similar analysis was used to compare taste ratings of chocolate milk and tasteless solution among the four groups.

Imaging data Based on our focus on brain regions involved in reward and motivation [4], we selected the amygdala, insula, putamen, caudate nucleus and orbitofrontal cortex (OFC) as our a priori regions of interest (ROI). We defined and localized ROIs specific to our tasks and

contrasts based on the orthogonal main effects of all participants in this study [34, 35]. To this end, contrasts of all participants were entered in a one-sample t-test and, for each contrast, a statistical map was calculated. To visualize brain activation in our anatomical ROIs only, we used an implicit anatomical mask that contained our bilateral ROIs, created with the WFU-Pick atlas toolbox (Wake Forest University, Winston-Salem, NC, USA). Using this implicit mask, we computed the statistical map of the one-sample T-test thresholded at $P<0.05$ family-wise error (FWE) whole brain corrected. Montreal Neurological Institute (MNI) coordinates of significantly activated peak voxels were used to create contrast-specific ROIs, by using spheres around the peaks with a radius of 10 mm (for insula, putamen, caudate nucleus and OFC) or 5 mm (for amygdala). Group comparisons were performed using a 2 by 2 factorial ANOVA design in SPSS [25], after extracting the individual raw beta-weights of each created ROI, using MarsBaR (MRC Cognition and Brain Sciences Unit, Cambridge, UK). *P*-values were calculated and reported for separate effects of GRS and BMI, and for the interaction between the two factors. A nominal *P*-value of 0.05 was set for these GRS and BMI effects.

RESULTS

CLINICAL CHARACTERISTICS

The four groups were comparable for age, handedness, daily smoking and menopausal status, as presented in Table 1. The proportion of premenopausal women that was scanned in the follicular phase of their menstrual cycle [36] was similar between groups. As expected, selection of participants from the four corners resulted in significant differences among groups in GRS and BMI ($P<0.001$). In keeping with a causal effect of BMI on these risk factors, women with high BMI had more unfavourable outcomes on HDL cholesterol and total/HDL cholesterol ratio irrespective of GRS. For fasting glucose a near significant ($P=0.057$) effect of GRS was observed irrespective of current BMI. Self-reported race was Caucasian (n=59) and Indo-Surinamese (n=1). However, projection of the genome-wide SNP data on a genome reference dataset [37] identified two individuals with a non-European ancestry, of which one matched the person identified through self-report.

BEHAVIOURAL RESULTS

Mean ratings of hunger and appetite prior to the scanning session were not affected by BMI, GRS or their interactions (Table 2). Figure 1A shows the mean palatability ratings of the high-calorie and low-calorie food pictures in the four groups. ANOVA revealed that there was no group by category interaction ($F[1, 56]=0.142$ $P=0.7$). Participants in all groups rated the low-calorie food pictures as more palatable than the high-calorie food pictures ($F[1, 56]=81.679$ $P<0.001$). Similarly,

no group by category interaction was observed for the ratings of chocolate milk and tasteless solution ($F[1, 55]=0.359$ $P=0.9$) (Figure 1B). Participants rated the taste stimuli as equally palatable ($F[1, 55]=0.196$ $P=0.7$). Furthermore, participants performed similarly on the image recognition test performed after the scan ($P>0.4$ for BMI, GRS and interaction), with mean percentages ± SD of images correctly recognized of 85.9 ± 10.0, 84.2 ± 6.0, 85.3 ± 7.9 and 86.8 ± 6.8 for the participants with low GRS/low BMI, low GRS/high BMI, high GRS/low BMI and high GRS/high BMI, respectively.

BRAIN RESPONSES TO FOOD PICTURES

In the overall group of participants, we observed a significant main effect for the contrast watching food vs. non-food pictures within our a priori anatomical ROIs, in particular the left amygdala, left OFC and bilateral insula (Table 3). Watching high-calorie vs. non-food pictures resulted in more activation of the left amygdala and left insula. Main effects of tasks in other regions of the brain are presented in Supplementary Table 1.

After extracting the mean activation of the task-specific ROIs created with MarsBaR, group comparisons showed no significant effects of GRS, BMI or its interactions on the mean activation in the left amygdala, OFC and bilateral insula to watching food vs. non-food pictures (Table 3), as analysed with factorial ANOVA. Similarly, no significant effects of BMI, GRS or its interactions were observed in the mean activation of the left amygdala and insula to watching high-calorie vs. non-food pictures.

BRAIN RESPONSES TO ANTICIPATION AND RECEIPT OF PALATABLE FOOD

Due to a technical problem during the scanning session, one participant (with low GRS/low BMI) could not complete the fMRI chocolate milk task. In the remaining group of women, we observed a significant main effect of chocolate milk anticipation vs. baseline in bilateral OFC (Table 3). The receipt of chocolate milk vs. baseline significantly activated bilateral amygdala, insula and right putamen. Main effects of tasks in other regions of the brain are presented in Supplementary Table 1. When contrasted to the tasteless solution, no main effects of chocolate milk receipt or anticipation were observed.

When comparing groups for mean activation in the task-specific ROI's, factorial ANOVA revealed that women with high GRS as compared to women with low GRS had significantly higher activation associated with the anticipation of chocolate milk vs. baseline in the right OFC, irrespective of BMI ($P=0.04$) (Table 3 and Figure 2A and B). The receipt of chocolate milk vs. baseline elicited higher activation in women with high BMI as compared to women with low BMI in bilateral amygdala, irrespective of GRS ($P<0.05$) (Figure 2C and D). No significant interactions between GRS and BMI were observed.

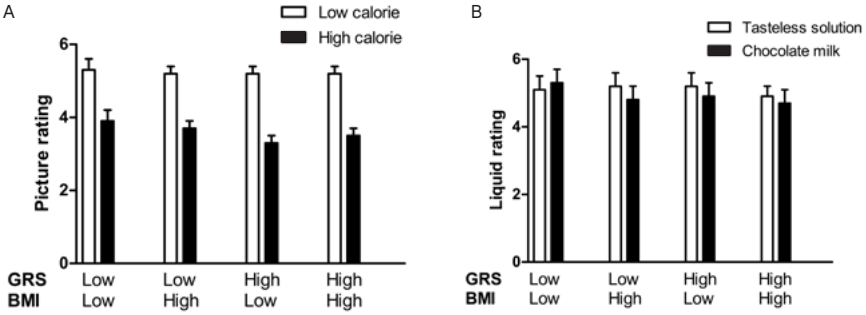

FIGURE 1 Appeal ratings of functional MRI stimuli
Mean scores ± SEM of self-reported appeal of low-calorie and high-calorie food pictures (A) and chocolate milk and tasteless solution (B) used in the functional MRI paradigms in women with low GRS/low BMI (n=16), low GRS/high BMI (n=12), high GRS/low BMI (n=15) and high GRS/high BMI (n=17). Data are analysed using mixed between-group (2 by 2 factors) within-group (2 food categories) ANOVA.

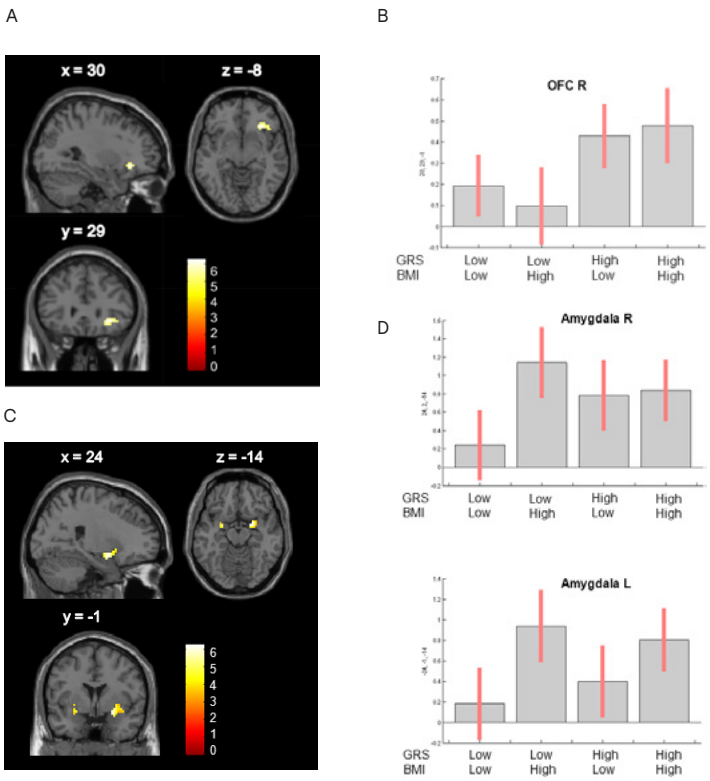

FIGURE 2 Main effects of functional MRI tasks and group differences among the four corners
Sagittal, axial and coronal slices showing the main effect of task (A) and group differences (B) in activation in response to the anticipation of chocolate milk vs. baseline in the right orbitofrontal cortex (OFC) (MNI 30 29 −8). Main effect of task (C) and group differences (D) in activation in response to chocolate milk receipt vs. baseline in the left amygdala (MNI −24 −1 −14) and right amygdala (MNI 24 2 −14). Left side of axial and coronal slices is the left side of the brain. X, Y and Z are the MNI space coordinates. Colour bar represents t statistics. Bars in figure B and D represent mean and 90% confidence intervals of contrast estimates (effect size, in arbitrary units) for women with low GRS/low BMI (n=15), low GRS/high BMI (n=12), high GRS/low BMI (n=15) and high GRS/high BMI (n=17). GRS, polygenic risk score; OFC, orbitofrontal cortex; R, right; L, left.

DISCUSSION

We investigated brain reward responses to the viewing of food pictures and to the anticipation and consumption of a palatable food stimulus in women with either a low or a high genetic predisposition to obesity, based on a polygenic risk score (GRS) of the 77 recently identified obesity-associated single nucleotide polymorphisms (SNPs) in European individuals [16]. To differentiate genetic effects that predispose to obesity from effects that are secondary to a high BMI, we further divided the study population into women with either a low or a high current BMI. This so-called 'four corners epidemiological approach' has been established previously [23-25] and used to identify a variety of factors that mediate the link between parental predisposition to hypertension and high blood pressure in the offspring.

In the current study, we observed that the anticipation of chocolate milk receipt elicited increased activation in the right orbitofrontal cortex (OFC) in women with high GRS for obesity as compared to women with low GRS for obesity, irrespective of current BMI. This finding suggests that an elevated response to the anticipation of palatable food in brain areas implicated in reward is a feature of the genetic predisposition to obesity. This finding fits with evidence from an earlier prospective study which demonstrated that greater OFC activation when viewing appetizing food pictures predicts future weight gain [8, 9]. Moreover, a study that used parental overweight to identify adolescents as having either a high or low genetic risk to obesity, demonstrated that individuals with high obesity risk have greater activation of reward regions, including the OFC, in response to palatable food receipt than individuals with low obesity risk [38]. In the Stice et al. study, however, high-risk participants had a slightly higher BMI than low-risk participants which makes it difficult to infer a causal relationship regarding reward region responsiveness and BMI. Furthermore, the use of parental occurrence of overweight to define familial risk to obesity in the offspring does not fully exclude effects of shared environmental factors unlike measured GRS as was done in our current study.

Evidence is emerging that multiple genetic variants associated with common obesity exert their effects on weight through the regulation of appetite and reward in the brain [16, 18]. Besides the genetic determinant with the strongest effect on BMI, the *FTO* gene, an association with processes in the central nervous system has been found for *ELAVL4*, *GRID1*, *CADM2*, *NRXNR*, *NEGR1* and *SCG3* [16]. Other SNPs are located near genes that have clearly shown involvement in the leptin-melanocortin pathway in the hypothalamus (*MC4R*, *BDNF*, *POMC* and *SH2B1*), as observed by studying monogenic forms of obesity [17, 18]. Although the recently identified obesity-associated loci together explain only a small amount (2.7%) of BMI variation [16], the usefulness of a multilocus GRS in studying appetitive mechanisms has already been demonstrated previously [39-41]. Specifically, whereas the effects of a 28-locus GRS on BMI

was mediated by lower satiety responsiveness in children [39], two other studies suggested mediating effects of uncontrolled and emotional eating behaviour, using risk scores comprising 32 and 90 BMI-related loci, respectively [40, 41]. Similar to our study, these observations provide evidence that a combined weighted GRS has the potential to identify mechanisms involved in food reward and appetite that contribute to the genetic predisposition to obesity.

A second major finding of our current study was an elevated response in bilateral amygdala in response to the receipt of chocolate milk in women with high BMI compared to women with low BMI, irrespective of GRS for obesity. This finding suggests that an *increased* amygdala response to palatable food receipt is secondary to overeating and/or weight gain. This result is at odds with our expectation based on previous studies that weight gain induces a *decreased* striatal response to palatable food receipt [11, 13]. These discrepant observations may reflect the multi-faceted aetiology of obesity, in which different brain regions implicated in the experience of eating may exert different effects to the receipt of palatable food. Whereas a decreased striatal response to food receipt may reflect the experience of lower reward due to neuro-adaptive changes caused by habitual overeating [13], an increased amygdala response to food receipt may reflect elevated responsiveness of regions involved in reward-based learning [42]. The latter, also known as conditioning, has shown to occur in response to repeated intake of high-calorie palatable foods [43]. It should be noted, however, that the increased amygdala response in our study was observed in response to *food consumption*, whereas conditioning, by definition, elicits responses to *cues* that signal the delivery of the food. A possible role for conditioning is nonetheless supported by several previous studies in which consumption of a palatable food stimulus also elicited *increased* rather than *decreased* activation in obese versus lean individuals in regions implicated in reward, attention and gustation, including the amygdala [7, 38, 44]. Thus, we propose that the receipt of small amounts of palatable foods may have conditioning effects similar to visual food cues. Together, our results suggest that an increased valuation of palatable food receipt may occur secondary to overeating and/or weight gain, which may further enhance weight gain in an environment dominated by the supply of high-calorie palatable food.

Contrary to our expectations, we did not observe significant effects of BMI, GRS or its interactions on the viewing of food pictures, or specifically high-calorie food pictures. It may be argued that, given the food appeal ratings, watching high-calorie as opposed to low-calorie food pictures failed to elicit sufficiently rewarding effects. However, watching food pictures and high-calorie food pictures in the overall group of women was associated with robust BOLD activation in the a priori ROIs, indicating that our paradigm was effective. A possible explanation for the absence of significant group differences in the food picture paradigm is lack of power. Our sample size was based on the

findings of significant BMI-related effects in previous published studies that included similar or even smaller sample sizes with comparable group differences in BMI [2-5], although we cannot rule out that these studies were underpowered for the BMI-related effect themselves.

Perhaps more concern is warranted for the power of the GRS. The currently identified obesity SNPs explain only a small percentage of the variance in BMI [16]. However, we again note that previous studies have shown potential effectiveness of polygenic risk scores based on fewer obesity-SNPs [39, 41] and that the power in our study was further enhanced compared to these previous studies by selecting individuals with extreme genotypes from a very large base population (n>11,000). Nevertheless, future research using larger sample sizes and risk scores comprising more obesity-related SNPs are warranted, as these may be able to detect more significant genetic effects on reward responses to visual food cues.

A final limitation of our study is that, when analysing the data of our chocolate milk experiment, robust reward responses in the total group of women were observed when the receipt and anticipation of chocolate milk were contrasted against a baseline BOLD activation, but not relative to the tasteless solution. This implicates that the group differences we observed are associated with the receipt and anticipation of a taste stimulus in general, but not specifically a high-calorie palatable taste stimulus. The absence of a significant main effect of chocolate milk when contrasted to the tasteless solution is most likely the result of a rewarding effect of the latter stimulus, which was originally designed to act as a neutral comparator [11]. This fits with our results of the food appeal ratings of the taste stimuli after the scan, which did not differ between chocolate milk and tasteless solution.

Strengths of our study are the use of a GRS based on the most recent findings of obesity-related SNPs [16] and the use of the four corner epidemiological model [23-25] which, by selecting individuals on extreme phenotypes and genotypes, allows to separate effects that predispose to obesity from effects secondary to increased BMI. Furthermore, the use of a measured GRS rather than parental characteristics to express genetic risk allowed us to study genetic effects independent of family environmental background.

In conclusion, genetic predisposition to obesity may act, at least in part, through elevated reward responsiveness to cues that signal palatable food receipt. In addition, habitual overeating and increased BMI itself may result in higher valuation of palatable food, which may result in even more overeating if the availability of energy dense palatable food is high. Our findings imply that interventions should focus on discouraging the intake of energy-dense palatable food in individuals from early age to prevent the development of conditioning to cues linked to the intake of these food types, especially in individuals with high genetic risk to obesity.

TABLE 1
Clinical characteristics

	Low GRS Low BMI (n=16)	Low GRS High BMI (n=12)	High GRS Low BMI (n=15)	High GRS High BMI (n=17)	*P*-value by ANOVA		
					GRS	BMI	Interaction
Age (y)	44.8 ± 6.3	47.0 ± 7.3	44.3 ± 7.4	47.1 ± 6.9	0.935	0.171	0.874
GRS	0.87 ± 0.05	0.87 ± 0.05	1.06 ± 0.05	1.05 ± 0.06	< 0.001	0.779	0.764
Weight (kg)	61.2 ± 6.6	89.7 ± 10.9	60.5 ± 4.8	92.7 ± 13.2	0.642	< 0.001	0.459
BMI (kg/m²)	20.9 ± 0.9	31.6 ± 3.7	20.4 ± 1.3	32.8 ± 4.8	0.636	< 0.001	0.289
Waist-hip ratio	0.78 ± 0.04	0.87 ± 0.05	0.78 ± 0.03	0.87 ± 0.06	0.744	< 0.001	0.726
Body fat (%)	27.8 ± 3.1	42.7 ± 2.9	27.1 ± 3.4	44.6 ± 4.7	0.573	< 0.001	0.208
Fasting glucose (mmol/L)	4.8 ± 0.4	4.8 ± 0.5	5.0 ± 0.4	5.1 ± 0.6	0.057	0.193	0.787
Total cholesterol (mmol/L)	4.9 ± 0.8	5.2 ± 0.8	4.9 ± 1.2	5.2 ± 1.3	0.869	0.410	0.567
HDL cholesterol (mmol/L)	1.9 ± 0.3	1.4 ± 0.5	1.9 ± 0.3	1.5 ± 0.6	0.413	< 0.001	0.937
LDL cholesterol (mmol/L)	2.8 ± 0.6	3.5 ± 0.9	2.7 ± 1.0	3.1 ± 1.0	0.611	0.042	0.398
Ratio total / HDL cholesterol	2.8 ± 0.8	4.0 ± 1.0	2.6 ± 0.9	4.0 ± 1.9	0.524	< 0.001	0.916
					P-value by chi-square		
Daily smoking (%)	2 (12.5)	1 (8.3)	2 (13.3)	0 (0)		0.5	
Right handedness (%)	15 (93.8)	9 (75)	13 (86.7)	15 (88.2)		0.7	
Premenopausal status (%)	11 (68.8)	7 (58.3)	11 (73.3)	9 (52.9)		0.6	
Follicular menstrual phase (%)	5 (45.5)	2 (28.6)	4 (36.4)	3 (33.3)		0.8	
Oral contraceptives use (%)	3 (18.8)	4 (33.3)	1 (6.7)	2 (11.8)		0.3	

Mean ± SD or n (%). All biochemical assessments are done in the fasted state. GRS, polygenic risk score; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

TABLE 2
VAS-scores of hunger and appetite ratings

	Low GRS Low BMI (n=16)	Low GRS High BMI (n=12)	High GRS Low BMI (n=15)	High GRS High BMI (n=17)	*P*-value by ANOVA		
					GRS	BMI	Interaction
Hunger	5.0 ± 2.8	3.6 ± 1.9	4.0 ± 2.2	3.6 ± 2.2	0.417	0.138	0.412
Fullness	3.5 ± 1.8	3.5 ± 2.5	2.7 ± 1.9	2.9 ± 1.8	0.199	0.839	0.839
Prospective food consumption	5.8 ± 1.6	4.3 ± 1.7	5.3 ± 1.4	5.3 ± 1.9	0.537	0.099	0.091
Desire for sweet food	3.1 ± 2.8	2.4 ± 2.3	2.7 ± 2.4	3.2 ± 2.2	0.813	0.876	0.341
Desire for savory food	5.1 ± 3.0	3.1 ± 2.4	4.6 ± 2.1	4.1 ± 2.7	0.707	0.069	0.294
Desire for fat	2.0 ± 2.2	1.1 ± 1.6	1.7 ± 1.9	1.3 ± 1.7	0.900	0.191	0.579

Mean ± SD. GRS, polygenic risk score; VAS, visual analogue scale

TABLE 3
Main effects of tasks in regions of interest and group comparisons

			Main effect		MNI			*P*-value by ANOVA		
Contrast	Region	Side	k	T	x	y	z	GRS	BMI	Interaction
Food vs. non-food pictures	Amygdala	L	2	5.05	-21	-4	-17	0.631	0.727	0.320
	Insula	L	17	7.01	-36	5	-14	0.434	0.705	0.699
	OFC	L	9	5.41	-6	62	-5	0.670	0.965	0.701
	Insula	R	2	4.91	39	8	-14	1.000	0.626	0.455
High-calorie vs. non-food pictures	Amygdala	L	4	5.49	-21	-4	-17	0.276	0.957	0.838
	Insula	L	6	5.88	-36	5	-14	0.572	0.582	0.862
Anticipation choco vs. baseline	OFC	L	1	4.84	-48	17	-8	0.711	0.605	0.580
	OFC	R	27	6.15	30	29	-8	0.040	0.935	0.891
Receipt choco vs. baseline	Amygdala	L	2	5.29	-24	-1	-14	0.716	0.013	0.192
	Insula	L	15	6.31	-36	-7	10	0.619	0.461	0.372
	Amygdala	R	16	6.27	24	2	-14	0.309	0.049	0.059
	Insula	R	31	7.65	39	-1	13	0.448	0.779	0.340
	Putamen	R	16	5.42	27	5	-5	0.831	0.242	0.574

Montreal Neurological Institute (MNI) coordinates of peak voxels activated in the total group of participants with threshold $P<0.05$ FWE whole brain corrected and visualized with an anatomical mask of a priori regions of interest; and group comparisons by factorial ANOVA. K, cluster size; T, T-statistic; GRS, polygenic risk score; L, left; R, right; OFC, orbitofrontal cortex; choco, chocolate milk.

SUPPLEMENTARY TABLE 1
Main effects of tasks whole brain

	Side	k	T	MNI x	MNI y	MNI z
Food vs. non-food pictures						
Occipital cortex and fusiform gyrus	L	893	13.4	-42	-82	-2
Occipital cortex	R	736	12.1	39	-70	-11
Posterior cingulate cortex	L	175	8.8	-6	-52	22
Postcentral gyrus	L	74	7.2	-42	-28	34
Insula	L	26	7.0	-36	5	-14
Superior parietal lobe	R	31	6.4	21	-61	58
Superior parietal lobe	L	71	6.0	-24	-58	58
Superior parietal lobe	L	9	5.4	-6	62	-5
Amygdala	L	2	5.1	-21	-4	-17
Superior frontal lobe	L	1	5.0	-18	32	43
Superior frontal lobe	L	2	4.9	-12	38	43
Insula	R	3	4.9	39	8	-14
High-calorie vs. non-food pictures						
Occipital cortex	L	903	13.8	-42	-79	-2
Occipital cortex	R	774	13.2	42	-76	-2
Posterior cingulate cortex	L	101	8.4	-6	-52	22
Superior parietal lobe	R	45	6.4	24	-58	58
Postcentral gyrus	L	58	6.1	-45	-28	34
Postcentral gyrus	R	22	6.0	60	-19	28
Insula	L	10	5.9	-36	5	-14
Superior parietal lobe	L	43	5.8	-24	-58	58
Amygdala	L	4	5.5	-21	-4	-17
Middle occipital cortex	R	2	5.2	27	-70	31
Superior occipital cortex	L	2	4.9	-24	-70	31
Anticipation chocolate milk vs. baseline						
Occipital cortex and fusiform gyrus	L/R	6018	19.0	-39	-67	-11
Precentral gyrus	L	75	7.2	-51	-1	43
Precentral gyrus and inferior frontal gyrus	R	282	6.7	51	8	43
Orbitofrontal cortex	R	32	6.2	30	29	-8
Supplementary motor area	R	30	5.6	3	11	55
Inferior parietal lobe	R	22	5.4	48	-34	40
Inferior frontal gyrus	L	25	5.4	-45	8	28
Middle temporal gyrus	R	7	5.2	48	-22	-8
Inferior frontal gyrus	L	2	5.1	-24	26	-8
Superior temporal gyrus	L	3	5.0	-51	14	-11
Cerebellum	L	2	5.0	-6	-79	-35
Inferior frontal gyrus	L	1	4.8	-60	11	13
Receipt chocolate milk vs. baseline						
Pre- and postcentral gyrus	L	640	11.4	-60	-22	22
Postcentral gyrus, rolandic operculum and insula	R	746	11.3	60	-16	19
Cerebellum	R	100	8.1	24	-70	-23
Supplementary motor area	L/R	73	6.5	6	5	55
Insula	L	21	6.3	-36	-7	10
Cerebellum	L	83	6.3	-18	-67	-23
Amygdala and putamen	R	20	6.3	24	2	-14
Middle temporal gyrus	L	42	6.1	-54	-67	4
Cerebellum	L	7	5.4	-42	-61	-29
Temporal lobe	R	1	5.4	45	-22	-14

Cerebellum	L	7	5.3	−15	−76	−35
Amygdala	L	2	5.3	−24	−1	−14
Temporal lobe	R	2	5.2	48	−16	−17
Superior frontal lobe	L	2	5.1	−18	−1	67

Montreal Neurological Institute (MNI) coordinates of peak voxels activated in the total group of participants with threshold $P<0.05$ FWE whole brain corrected. K, cluster size; T, T-statistic; L, left; R, right

REFERENCES

1
Berthoud HR. Metabolic and hedonic drives in the neural control of appetite: who is the boss? Curr Opin Neurobiol. 2011;21(6):888-96.

2
Ten Kulve JS, Veltman DJ, van Bloemendaal L, Barkhof F, Drent ML, Diamant M, et al. Liraglutide Reduces CNS Activation in Response to Visual Food Cues Only After Short-term Treatment in Patients With Type 2 Diabetes. Diabetes Care. 2015.

3
van Bloemendaal L, Ijzerman RG, Ten Kulve JS, Barkhof F, Konrad RJ, Drent ML, et al. GLP-1 receptor activation modulates appetite- and reward-related brain areas in humans. Diabetes. 2014;63(12):4186-96.

4
Pursey KM, Stanwell P, Callister RJ, Brain K, Collins CE, Burrows TL. Neural responses to visual food cues according to weight status: a systematic review of functional magnetic resonance imaging studies. Front Nutr. 2014;1:7.

5
Stoeckel LE, Weller RE, Cook EW, III, Twieg DB, Knowlton RC, Cox JE. Widespread reward-system activation in obese women in response to pictures of high-calorie foods. Neuroimage. 2008;41(2):636-47.

6
Killgore WD, Young AD, Femia LA, Bogorodzki P, Rogowska J, Yurgelun-Todd DA. Cortical and limbic activation during viewing of high- versus low-calorie foods. Neuroimage. 2003;19(4):1381-94.

7
Stice E, Spoor S, Bohon C, Veldhuizen MG, Small DM. Relation of reward from food intake and anticipated food intake to obesity: a functional magnetic resonance imaging study. J Abnorm Psychol. 2008;117(4):924-35.

8
Yokum S, Ng J, Stice E. Attentional bias to food images associated with elevated weight and future weight gain: an fMRI study. Obesity (Silver Spring). 2011;19(9):1775-83.

9
Stice E, Burger KS, Yokum S. Reward Region Responsivity Predicts Future Weight Gain and Moderating Effects of the TaqIA Allele. J Neurosci. 2015;35(28):10316-24.

10
van Bloemendaal L, Veltman DJ, Ten Kulve JS, Groot PF, Ruhe HG, Barkhof F, et al. Brain reward-system activation in response to anticipation and consumption of palatable food is altered by glucagon-like peptide-1 receptor activation in humans. Diabetes Obes Metab. 2015;17(9):878-86.

11
Stice E, Spoor S, Bohon C, Small DM. Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. Science. 2008;322(5900):449-52.

12
Davis JF, Tracy AL, Schurdak JD, Tschop MH, Lipton JW, Clegg DJ, et al. Exposure to elevated levels of dietary fat attenuates psychostimulant reward and mesolimbic dopamine turnover in the rat. Behav Neurosci. 2008;122(6):1257-63.

13
Stice E, Yokum S, Blum K, Bohon C. Weight gain is associated with reduced striatal response to palatable food. J Neurosci. 2010;30(39):13105-9.

14
Stunkard AJ, Foch TT, Hrubec Z. A twin study of human obesity. JAMA. 1986;256(1):51-4.

15
Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet. 2010;42(11):937-48.

16
Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature. 2015;518(7538):197-206.

17
Albuquerque D, Stice E, Rodriguez-Lopez R, Manco L, Nobrega C. Current review of genetics of human obesity: from molecular mechanisms to an evolutionary perspective. Mol Genet Genomics. 2015;290(4):1191-221.

18
van der Klaauw AA, Farooqi IS. The hunger genes: pathways to obesity. Cell. 2015;161(1):119-32.

19
van der Klaauw AA, von dem Hagen EA, Keogh JM, Henning E, O'Rahilly S, Lawrence AD, et al. Obesity-associated melanocortin-4 receptor mutations are associated with changes in the brain response to food cues. J Clin Endocrinol Metab. 2014;99(10):E2101-E6.

20
Karra E, O'Daly OG, Choudhury AI, Yousseif A, Millership S, Neary MT, et al. A link between FTO, ghrelin, and impaired brain food-cue responsivity. J Clin Invest. 2013;123(8):3539-51.

21
Heni M, Kullmann S, Veit R, Ketterer C, Frank S, Machicao F, et al. Variation in the obesity risk gene FTO determines the postprandial cerebral processing of food stimuli in the prefrontal cortex. Mol Metab. 2014;3(2):109-13.

22
Belsky DW, Moffitt TE, Sugden K, Williams B, Houts R, McCarthy J, et al. Development and evaluation of a genetic risk score for obesity. Biodemography Soc Biol. 2013;59(1):85-100.

23
Noon JP, Walker BR, Webb DJ, Shore AC, Holton DW, Edwards HV, et al. Impaired microvascular dilatation and capillary rarefaction in young adults with a predisposition to high blood pressure. J Clin Invest. 1997;99(8):1873-9.

24
Harrap SB, Dominiczak AF, Fraser R, Lever AF, Morton JJ, Foy CJ, et al. Plasma angiotensin II, predisposition to hypertension, and left ventricular size in healthy young adults. Circulation. 1996;93(6):1148-54.

25
Watt GC, Harrap SB, Foy CJ, Holton DW, Edwards HV, Davidson HR, et al. Abnormalities of glucocorticoid metabolism and the renin-angiotensin system: a four-corners approach to the identification of genetic determinants of blood pressure. J Hypertens. 1992;10(5):473-82.

26
Boomsma DI, de Geus EJ, Vink JM, Stubbe JH, Distel MA, Hottenga JJ, et al. Netherlands Twin Register: from twins to twin families. Twin Res Hum Genet. 2006;9(6):849-57.

27
Willemsen G, Vink JM, Abdellaoui A, den BA, van Beek JH, Draisma HH, et al. The Adult Netherlands Twin Register: twenty-five years of survey and biological data collection. Twin Res Hum Genet. 2013;16(1):271-81.

28
Boomsma DI, Willemsen G, Sullivan PF, Heutink P, Meijer P, Sondervan D, et al. Genome-wide association of major depression: description of samples for the GAIN Major Depressive Disorder Study: NTR and NESDA biobank projects. Eur J Hum Genet. 2008;16(3):335-42.

29
Willemsen G, de Geus EJ, Bartels M, van Beijsterveldt CE, Brooks AI, Estourgie-van Burk GF, et al. The Netherlands Twin Register biobank: a resource for genetic epidemiological studies. Twin Res Hum Genet. 2010;13(3):231-45.

30
Schroevers MJ, Sanderman R, van SE, Ranchor AV. The evaluation of the Center for Epidemiologic Studies Depression (CES-D) scale: Depressed and Positive Affect in cancer patients and healthy reference subjects. Qual Life Res. 2000;9(9):1015-29.

31
Van Strien JW. Classificatie van links- en rechtshandige proefpersonen. Nederlands Tijdschrift voor de Psychologie en Haar Grensgebieden. 1992;47:88-92.

32
Doornweerd S, Ijzerman RG, Van der Eijk L, Neter JE, van DJ, van der Ploeg HP, et al. Physical activity and dietary intake in BMI discordant identical twins. Obesity (Silver Spring). 2016.

33
Ten Kulve JS, Veltman DJ, van Bloemendaal L, Groot PF, Ruhe HG, Barkhof F, et al. Endogenous GLP1 and GLP1 analogue alter CNS responses to palatable food consumption. J Endocrinol. 2016;229(1):1-12.

34
Friston KJ, Rotshtein P, Geng JJ, Sterzer P, Henson RN. A critique of functional localisers. Neuroimage. 2006;30(4):1077-87.

35
Kriegeskorte N, Simmons WK, Bellgowan PS, Baker CI. Circular analysis in systems neuroscience: the dangers of double dipping. Nat Neurosci. 2009;12(5):535-40.

36
Dreher JC, Schmidt PJ, Kohn P, Furman D, Rubinow D, Berman KF. Menstrual cycle phase modulates reward-related neural function in women. Proc Natl Acad Sci U S A. 2007;104(7):2465-70.

37
Abdellaoui A, Hottenga JJ, de KP, Nivard MG, Xiao X, Scheet P, et al. Population structure, migration, and diversifying selection in the Netherlands. Eur J Hum Genet. 2013;21(11):1277-85.

38
Stice E, Yokum S, Burger KS, Epstein LH, Small DM. Youth at risk for obesity show greater activation of striatal and somatosensory regions to food. J Neurosci. 2011;31(12):4360-6.

39
Llewellyn CH, Trzaskowski M, van Jaarsveld CH, Plomin R, Wardle J. Satiety mechanisms in genetic risk of obesity. JAMA Pediatr. 2014;168(4):338-44.

40
Konttinen H, Llewellyn C, Wardle J, Silventoinen K, Joensuu A, Mannisto S, et al. Appetitive traits as behavioural pathways in genetic susceptibility to obesity: a population-based cross-sectional study. Sci Rep. 2015;5:14726.

41
Cornelis MC, Rimm EB, Curhan GC, Kraft P, Hunter DJ, Hu FB, et al. Obesity susceptibility loci and uncontrolled eating, emotional eating and cognitive restraint behaviors in men and women. Obesity (Silver Spring). 2014;22(5):E135-E41.

42
Baxter MG, Murray EA. The amygdala and reward. Nat Rev Neurosci. 2002;3(7):563-73.

43
Burger KS, Stice E. Elevated energy intake is correlated with hyperresponsivity in attentional, gustatory, and reward brain regions while anticipating palatable food receipt. Am J Clin Nutr. 2013;97(6):1188-94.

44
Boutelle KN, Wierenga CE, Bischoff-Grethe A, Melrose AJ, Grenesko-Stevens E, Paulus MP, et al. Increased brain response to appetitive tastes in the insula and amygdala in obese compared with healthy weight children when sated. Int J Obes (Lond). 2015;39(4):620-8.