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ORIGINAL ARTICLE

Acute stress and food-related reward activation in the brain during food choice during eating in the absence of hunger

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Background: Stress results in eating in the absence of hunger, possibly related to food reward perception.
Hypothesis: Stress decreases food reward perception.
Aim: Determine the effect of acute stress on food choice and food choice reward-related brain activity.
Subjects: Nine females (BMI = 21.5 ± 2.2 kg/m^2, age = 24.3 ± 3.5 years).
Procedure: Fasted subjects came twice to randomly complete either a rest or stress condition. Per session, two functional MRI scans were made, wherein the subjects chose the subsequent meal (food images). The rewarding value of the food was measured as liking and wanting. Food characteristics (for example, crispiness, fullness of taste and so on), energy intake, amount of each macronutrient chosen, plasma cortisol and Visual Analog Scale (VAS) hunger and satiety were measured.
Results: Fasted state was confirmed by high hunger (80 ± 5 mm VAS). Breakfast energy intake (3 ± 1 MJ) and liking were similar in all conditions. Wanting was lower postprandially ($\Delta = -0.3$ items/category, $P<0.01$). Breakfast decreased hunger (−42 mm VAS, $P<0.01$). Postprandially, energy intake (−1.1 MJ), protein intake (−14.7 g) and carbohydrate intake (−32.7 g all $P<0.05$) were lower. Fat intake was not different (−7.3, $P=0.4$). Putamen activity was not lower postprandially. Cortisol levels were increased in the stress condition (Area under the curve of cortisol: $\Delta AUC = +2.2 \times 10^4$ nmol min^{-1} l^{-1}, $P<0.05$). Satiety was lower after breakfast (−8 mm VAS, $P<0.01$). Postprandial energy intake, protein intake and carbohydrate intake were relatively higher compared with the rest condition, resulting from more choice for crispiness and fullness of taste ($P<0.05$). Brain activation was reduced in reward areas: amygdala, hippocampus and cingulate cortex (AUC = −13.33, −1.34, −2.56% blood oxygen level dependent (BOLD) s for choosing breakfast and AUC = −9.31, −1.25, −2.34%BOLD s <0.05 for choosing the second meal). Putamen activation was decreased postprandially (AUC = −1.2%BOLD s, $P<0.05$).
Conclusion: Reward signaling and reward sensitivity were significantly lower under stress, coinciding with increased energy intake from food choice for more crispiness and fullness of taste. The changes in putamen activation may reflect specifically decreased reward prediction sensitivity.
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Keywords: stress; eating in the absence of hunger; fMRI; neuroscience; food reward

Introduction

The currently rising obesity epidemic in the developed world is drawing increasing attention,[1] especially as visceral (or central) obesity is a key factor in the development of the metabolic syndrome.[2,3] It has been shown that increased stress indices coincide with the increased prevalence of obesity and the metabolic syndrome.[4,5] Stress is indicated by increased activity of the hypothalamus pituitary adrenal axis and is represented by elevated plasma cortisol levels.[6,7] It has been shown that viscerally obese subjects often have increased cortisol levels compared with normal weight subjects.[8] Furthermore, an interaction between cortisol and leptin has been shown; the obesogenic effects of

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leptin deficiency in rats can be reversed by subsequent adrenalectomy.[9] This indicates that both leptin and cortisol signaling simultaneously contribute to food intake regulation. Other studies have indicated that also insulin levels are positively related to cortisol levels and that insulin and cortisol have antagonistic effects on each other in terms of feeding behavior and body weight.[10–12] Furthermore, it was shown that cortisol and insulin co-determine the macronutrient intake in rats, such that higher insulin levels were associated with higher lard intake in rats.[13]

Additionally, other studies suggest that high cortisol levels leads to altered food choice[14–16] and stress is thought to result in food choice for items with a higher content of fat and sweet, which are perceived as highly rewarding.[15,17–20] Over all, this is a striking evidence that stress influences food choice through various hormonal pathways.

Eating behavior that may be influenced by stress is divided into two components: the first component is homeostatic eating, which relates to hunger and satiety, and ultimately to energy balance and steady body weight. The second component is non-homeostatic eating, which is influenced by food reward, and is observed as eating in the absence of hunger.[21] Westerterp and Speakman[22] performed an extensive meta analyses on energy expenditure data of cohorts around the world. They showed that the energy expenditure of adults was related to factors such as BMI, but did not decrease at all between 1988 and 2006.[22] This suggests that the recent rise in obesity may result from non-homeostatic eating over long periods of time, rather than a lack of physical exercise. Furthermore, we showed that stress leads to eating in the absence of hunger in subjects with high disinhibition scores.[23] This suggests that stress interferes with non-homeostatic pathways that are involved in food intake regulation, such as food reward signaling, resulting in a shift of food choice toward foods with high fat and carbohydrate content.[23] Taken together, we hypothesize that stress significantly decreases the food reward, leading to non-homeostatic eating in the absence of hunger, with food choices that result in higher energy intakes.

The rewarding value of food can be described as consisting of two components: liking, which is the hedonic preference for a given food item and wanting, which is the motivation to obtain the food item.[24] The combination of liking and wanting, defines the rewarding value of a given item and thereby its specific maximum perceived food reward. At the level of the central nervous system, it has been shown that reward activates dopamine-mediated signaling in key areas of the brain. In studies that investigated food reward specifically with various paradigms, using food images, smells and tastes, brain areas involved were the amygdala, striatum, hippocampus anterior cingulate cortex and orbitofrontal cortex.[25–30] Previous studies have shown that dopamine release in these brain areas is dependent on satiety, but also on BMI,[31,32] which by itself has been inversely correlated with dopamine-mediated signaling.[32] This indicates that dopamine reward signaling may be involved in food intake regulation. Finally, several reports show interactions between dopamine reward systems and cortisol, or corticotropin releasing factor,[33–37] thus suggesting that a state of endocrinological stress may directly lead to dramatic changes in food reward signaling.

We hypothesize that acute stress reduces signaling in food reward circuits, thereby leading to eating in the absence of hunger. To test the hypothesis that stress reduces the rewarding effect of food in general, we aimed to determine the effects of acute stress on food reward-related brain activation that was induced by food choice.

Methods

Subjects

The study was approved by the Medical Ethical Committee of Maastricht University, and informed, written consent was obtained from all subjects. Inclusion criteria were female, normal body weight (BMI 19–25), right handedness. Exclusion criteria were recent dieting, a personal or familial history of psychiatric disorder or intrauterine contraceptives. Fourteen test subjects were screened and 10 of them were included with the following characteristics: age = 24.1 ± 1.1, BMI = 21.5 ± 0.7, Three Factor Eating Questionnaire,[38] dietary restraint = 8.4 ± 1.1, disinhibition = 4.2 ± 0.5, emotional eating = 4.6 ± 0.9, age = 24.3 ± 1.2, State Trait Anxiety Inventory-2 (trait) = 37.8 ± 6.2.

Sessions

Subjects came to the university twice in the fasted state. Randomly two sessions were completed, one in the rest condition and one in the stress condition, each at least 1 week apart. To create stress versus rest, an unsolvable versus solvable mathematical test was given before each scan. This test has been described and validated before.[23,39] A schedule of how the sessions were executed is given in Figure 1. Each session included five questionnaires and five blood samples, two functional MRI (fMRI) scans and two meals (breakfast after the first scan and a postprandial meal after the second scan) that were chosen from food items shown within the scanner (Table 1). The meals were offered immediately after the questionnaires and blood samples were obtained quickly after the scan.

Visual Analog Scales

To determine the effect of the breakfast and a postprandial meal, Visual Analog Scales (VAS) for hunger and satiety were taken five times: baseline > 20 min after placing the cannula, before the first scan, immediately after the first scan before breakfast, before the second scan and immediately after the second scan before the second meal. The VAS questionnaires consisted of 100 mm lines, anchored with 'not at all' at the far left 'extremely' at the far right. Questions asked were 'How hungry are you?' 'How full do you feel?' 'How satiated

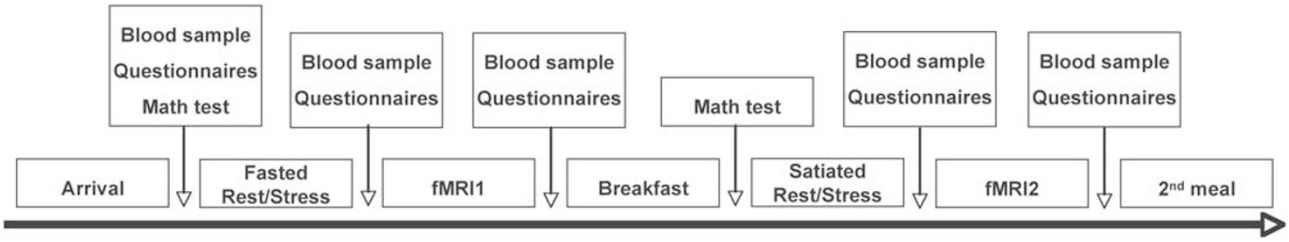

Figure 1 Schedule of the activities that subjects conducted per test session.

Table 1 Food characteristics of the food items used in the experiment

Item name	*Crispiness*	*Creami-ness*	*Fullness of taste*	*Sweet*	*Sour*	*Salty*	*Bitter*	*Energy (kJ)*	*Protein (g)*	*Carb (g)*	*Fat (g)*	*En. density (kJ g^{-1})*
Bread												
Brown bread	1.13	1.25	1.63	1.63	1.25	1.75	1.38	262.00	3.33	15.79	1.12	10.48
White bread	1.25	1.38	1.25	1.38	1.13	2.13	1.13	286.00	3.40	17.96	1.02	11.44
Soft white roll	1.13	1.75	1.75	1.75	1.38	2.38	1.13	468.40	6.51	37.17	3.50	12.46
Soft brown roll	1.13	1.88	2.00	1.63	1.13	2.00	1.25	420.00	6.65	31.57	2.45	10.50
Croissant	1.75	2.00	2.75	2.88	1.13	1.63	1.13	794.00	6.44	35.21	18.41	19.85
White hard roll	2.50	1.25	1.75	1.63	1.25	2.38	1.25	445.20	5.67	36.40	1.47	11.01
Wasa cracker	3.00	1.00	1.63	1.25	1.25	1.63	1.25	157.56	0.63	4.62	0.11	13.50
Whole wheat roll	1.63	1.50	2.50	1.38	2.00	1.88	1.38	573.30	5.67	36.61	2.45	11.47
Fillings												
Brie cheese	1.13	3.00	2.88	1.13	1.25	2.50	1.63	605.60	6.72	0.00	13.28	15.14
Gouda cheese	1.00	2.75	2.88	1.25	1.50	2.38	1.25	405.08	7.99	0.00	10.36	15.74
Salami	1.00	2.13	2.63	1.25	1.63	2.88	1.38	310.80	3.90	0.02	6.60	15.54
Ham	1.38	1.38	2.38	1.25	1.25	2.75	1.13	114.60	4.35	0.40	1.70	5.73
Sandwich spread	2.00	2.25	2.63	1.13	3.00	1.88	1.63	473.00	0.80	7.00	9.50	9.46
Peanut butter	1.13	3.00	2.63	1.88	1.38	2.50	1.25	402.90	3.30	1.50	8.70	26.86
Chocolate sprinkles	2.25	1.88	2.75	3.00	1.00	1.00	1.25	362.00	1.22	14.00	2.90	18.10
Apricot jam	1.00	2.00	2.25	3.00	2.13	1.00	1.50	255.75	0.05	15.75	0.00	10.23
Drinks												
Water	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00
Orange juice	1.00	1.00	2.25	1.75	2.88	1.13	1.88	342.00	1.75	21.50	0.00	1.71
Chocolate milk	1.00	2.63	2.88	3.00	1.00	1.13	1.50	754.00	8.75	30.00	6.25	3.77
Milk	1.00	1.88	2.38	1.88	1.00	1.50	1.13	388.00	9.00	11.50	3.75	1.94
Coca Cola	1.00	1.00	2.25	3.00	1.88	1.13	1.13	582.12	0.00	34.98	0.00	1.76
Buttermilk	1.00	1.50	2.25	1.13	3.00	1.00	1.13	266.00	8.50	6.50	1.00	1.33
Apple juice	1.00	1.00	2.00	2.75	2.38	1.00	1.38	316.00	0.25	26.25	0.00	1.58
Aperitif bitter	1.00	1.00	2.38	1.63	1.75	1.13	3.00	290.00	0.00	16.50	0.00	2.90
Deserts												
Apple	2.38	1.00	1.75	2.50	2.63	1.00	1.38	351.90	0.48	14.16	0.00	2.07
Orange	2.00	1.00	1.88	2.38	1.88	1.13	1.25	396.00	1.20	12.72	0.00	1.98
Banana	1.13	2.38	2.50	2.75	1.00	1.00	1.13	750.00	1.08	18.36	0.18	3.75
Vanilla cake	1.38	1.75	2.38	2.88	1.00	1.13	1.13	614.95	1.96	17.78	8.12	17.57
Vanilla custard	1.00	2.88	2.25	3.00	1.00	1.25	1.13	490.00	3.25	16.38	3.50	3.70
Chocolate mousse	1.00	2.75	2.88	2.88	1.50	1.00	1.88	1315.00	6.63	31.00	7.88	7.43
Chocolate pudding	1.00	2.75	2.75	3.00	1.00	1.13	1.13	527.50	3.63	17.50	3.65	4.00
Yoghurt	1.13	2.50	2.75	2.88	2.00	1.00	1.25	523.75	5.50	20.00	4.00	49.50
Snacks												
Salted peanuts	2.75	1.38	2.38	1.50	1.13	3.00	1.63	1035.60	8.40	3.60	15.60	26.00
Tuc cracker	3.00	1.75	2.00	1.63	1.25	2.88	1.13	586.88	2.40	19.50	6.60	20.55
Gummi bears	1.25	1.13	1.50	3.00	1.25	1.13	1.25	390.15	0.30	21.90	0.00	8.67
Cigarette	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Potato chips	3.00	1.25	2.13	1.25	1.25	2.88	1.25	904.80	2.60	21.60	13.60	22.80
Chocolate	2.25	2.63	2.50	2.88	1.13	1.13	1.25	841.88	2.25	21.00	12.00	22.53
Chocolate, white	1.75	2.75	2.75	3.00	1.13	1.13	1.25	900.38	2.25	21.00	13.13	23.66
Licorice	2.25	1.25	2.50	2.63	1.25	2.38	1.38	221.85	2.00	15.40	0.00	14.79

All items are shown in their respective food groups (bread, fillings, drinks, desserts and snacks) with the macronutrient content (g), engery content (kJ), energy density (kJ g^{-1}), structural properties and taste properties (arbitrary units).

do you feel?' 'How thirsty are you?' and 'How large is your desire to eat?'

Hormones
At the beginning of the test session, a cannula was placed in the antecubital vein, which was kept there for the duration of the session. After each time that the questionnaires were filled out, a blood sample for hormone measurements was drawn (10 ml). Thus, in total five blood samples were obtained starting >20 min after placing the cannula. These samples were each divided over two tubes: 6 ml was transferred into a serum tube (BD Vacutainer with separation gel, 8.5 ml, Franklin Lakes, NJ, USA). Blood serum was obtained by centrifugation (4 °C, 3000 r.p.m., 10 min). The remaining 4 ml were transferred into a plasma tube (BD Vacutainer with EDTA, 10 ml). Blood plasma was obtained by centrifugation (4 °C, 3000 r.p.m., 20 min). All samples were frozen and stored at −80 °C until further analysis. Serum cortisol levels for stress were determined by radio immuno assay (at Département des Sciences Fonctionnelles, B41-Physiologie de Reproduction, Liège, Belgium). Both leptin and insulin were determined in our own laboratory from the plasma samples, by means of radio immuno assay according to the manufacturer's instructions (Human insulin-specific and human leptin-specific RIA kit, Linco Research, St Charles, MO, USA).

Rewarding value
Rewarding value was determined as liking and wanting. To obtain data on the rewarding value of food and food intake, liking and wanting were analyzed as follows: the food items used in this study were ranked based on their relative liking in a separate set of experiments.[40] In our study, the liking scores refer to the average of the predetermined liking scores of all the chosen items for one meal. To determine these scores, all food items were shown to the same subjects in random pairs. Each food item was shown paired with all other items within the category and subsequently the items were additionally paired between the categories. By selecting the preferred items from each pair, a relative ranking was obtained for all items.[40]

To determine which food was wanted by our subjects, food items were chosen inside the scanner. These items had to be eaten immediately after the scan. The choice for the respective item together with the average number of items chosen therefore reflects wanting.

Food characteristics
To determine the characteristics of the selected food, the structural characteristics of each food item were determined by a separate 10-subject taste panel. All food items were offered individually and in randomized order. Water was provided to avoid cross tasting. The taste dimensions that were tested were crispiness, creaminess, fullness of taste, sweet, sour, salty and bitter. Fullness of taste was defined by instructing the test panel to consider how strong the taste of a given food item was as well as how much this taste filled the mouth. All dimensions were rated on a seven-point scale, ranging from 'not at all' to 'extremely' for each food item and each dimension. The average ratings were calculated and used in the subsequent analyses. Furthermore, macronutrient composition, total energy content and the average energy density of the chosen food were calculated (Table 1).

Functional MRI
The subjects were placed in supine position in a Siemens Magnetom Allegra, with the standard one-channel head coil. The subjects position was confirmed with T1 weighted scout images. In the fMRI scanner, the subjects were successively presented with five categories of food items: bread, fillings, drinks, desserts and snacks (Table 1). The subjects knew all items from a similar experiment outside the scanner, as well as the quantity that they were offered. For each category, a menu of eight images of the available food items was shown for ten seconds. The subjects were asked to choose between zero and up to two items from each of these menus to create their own meals, and they were instructed that those items had to be eaten completely immediately after the scan. To select the desired items, an MRI compatible joystick was used, which was fixed to the scan table at the waist at an angle that allowed comfortable usage. It was confirmed that the joystick was easily reached with the right hand to move the mouse pointer, and with the left hand to press a button to select the item. Before starting the food choices, subject had been in the MRI for 15 min, performing another behavioral paradigm. The food selection was preceded with 16 s gap (fixation cross), followed by written instruction regarding the subsequent food choice, which was followed by another 16 s gap. Food choice displays were separated by 16 s gaps. fMRI images were acquired throughout the session using a standard T2* weighted protocol to obtain blood oxygen level-dependent (BOLD) T2* signal (TR = 2 s, TE = 25 ms, Flip angle = 90°, matrix = 64 × 64, voxel size 3.5 mm × 3.5 mm × 3.5 mm).

Data analysis
Hormone level measurements and behavioral data were analyzed using factorial ANOVA with or without repeated measures, depending on the variables that were included. Pearson correlations were made per condition. With the exception of the fMRI image data, all data were analyzed using Microsoft Excel and SPSS 16. For cortisol data, areas under the curve were calculated using the trapezoid method.

The fMRI data were imported into BrainVoyager QX (Brain Innovation BV, Maastricht, The Netherlands). To preprocess the functional data, slice scan time correction with cubic spline interpolation, motion correction with trilinear interpolated motion estimation and subsequent sinc interpolation, and temporal high pass filtering with a window of 5 cycles was applied.

One out of the 10 subjects scanned had to be excluded from analysis because of excessive movement during the scan.

The functional data were aligned to each subjects' own 1 mm isovoxel high resolution T1 weighted anatomical scan. First, the head tissues were digitally removed from the brain, to optimize the performance of BrainVoyager's auto alignment algorithm. Second the auto alignment was performed and corrected manually under visual inspection, if necessary. Finally, all images were transformed into the Talairach coordinate system[41] using the standard procedure in BrainVoyager. Statistical analyses were superimposed on a group average anatomical brain image.

To analyze the brain activation, predictors for general linear model (GLM) analysis were created: One predictor with a duration of 10 s was created for each presentation of the menu from which the food had to be chosen. The predictors were modeled using the standard canonical two-gamma hemodynamic response function. Two predictor types were defined: menu presentation from which something was chosen, and menu presentation from which nothing was chosen. Group GLMs were performed, including the measurements for breakfast selection and the second meal selection (postprandially) in the rest and the stress condition. Group contrasts were used to compare activations in whole brain images and a functional voxel cluster threshold of $n = 4 \times 27\,\mathrm{mm}^3$ was set.

Anatomical regions of interest (ROI) were determined from literature[25–30] and generated bilaterally. The regions included the anterior cingulate cortex, amygdala, hippocampus, hypothalamus and putamen. Group contrasts for choosing something versus choosing nothing, revealed significantly higher activation in the right frontal cortex when something was chosen (Figures 3a–c, False discovery rate (FDR) corrected $P<0.05$). Additionally, it was found that signal in all ROIs was higher on average in case that food items were chosen, therefore, the analyses of reward-related activation were performed using the signal when food items were chosen. To determine functionally relevant ROI shapes, group contrasts were made for high wanting versus low wanting, using the predetermined ROI definitions as a mask. Subsequently, the significantly active voxels from this contrast were exported as the new ROI definitions.

The average percent BOLD change was extracted using event-related averaging. Events were defined as image presentations in which a choice was made for an item. The data that were extracted from the corrected ROI was imported into Microsoft Excel. Subsequently, the data were normalized to time $t=0$ (the moment that the food menu was shown). The averages of all measurements were plotted per ROI and the presence of hemodynamic response curve in the BOLD signal was visually confirmed. The area under the curve from the image presentation start until 12 s ($t=0$ to 12) was calculated using the trapezoid method and further analyzed using SPSS 16 using ANOVA repeated measures.

Results

The fasted state before the first scan was confirmed by low VAS scores for satiety and fullness, and high scores for hunger, thirst and desire to eat. Eating the self-selected breakfast lead to significantly decreased hunger, thirst and desire to eat, and to increased satiety and fullness for the remainder of the session (Table 2, VAS, $P<0.01$).

To compare the rewarding value of the food that was chosen within the session, the subjective preference was determined in terms of liking and wanting. Average predetermined relative liking scores for the food items did not differ between the stress and the rest condition, and between the fasted and the satiated (postprandial) condition. However, the quantitative wanting for food, measured as the number of items chosen, was significantly decreased after breakfast (Table 2, rewarding value, $P<0.01$ in the rest condition and $P<0.02$ in the stress condition).

The acute stress as induced by the mathematical test was confirmed with cortisol measurements: cortisol levels were significantly higher, after the unsolvable compared with the solvable math test (Table 2, cortisol, $P<0.05$). Leptin and insulin levels were not significantly increased in the stress condition compared with the rest condition (Table 2) and they were not correlated with cortisol levels.

The effect of stress on hunger and satiety was analyzed from the VAS scores for hunger and satiety. Subjects showed significantly lower scores for satiety after breakfast in the stress condition than in the rest condition, whereas the scores for hunger were equal in both conditions. The average predetermined liking of the food items chosen during stress was not different from the rest condition (liking). Likewise, the number of food items chosen (wanting) was no different in the stress condition compared with the rest condition. Fat intake was not different during the postprandial second meal compared with breakfast in both the stress and the rest condition (Table 2, n.s.). However, the energy intake, protein intake and carbohydrate intake were not decreased postprandially in the stress condition. Thus, carbohydrate and protein intake were relatively higher in the postprandial state (in the absence of hunger) in the stress condition compared with the rest condition. Regarding the food characteristics, first subjects selected food items with higher crispiness in the stress versus rest condition (Table 2, interaction effect $P=0.043$). Second, their preference for fullness of taste was decreased postprandially in the rest condition, but not in the stress condition (Table 2, interaction effect $P=0.024$) and finally, subjects tended to choose more energy dense food items in the absence of hunger in the stress condition compared with the rest condition (Table 2, interaction effect $P<0.06$). Postprandial energy intake was positively correlated with the cortisol levels in the rest condition and in the stress condition ($R=0.697$ and 0.695, both $P<0.04$).

Table 2 Behavioral, hormone, food and fMRI ROI data of nine female subjects

	Rest condition			*Stress condition*			P *time* condition*
	Fasted	*Satiated*	P	*Fasted*	*Satiated*	P	
Hormones							
Cortisol (AUC mmol l^{-1} min^{-1})	111.92 ± 9.26			133.73 ± 16.33			<0.05
Insulin (AUC mmol l^{-1} min^{-1})	6.78 ± 0.99			7.03 ± 1.21			n.s.
Leptin (AUC mmol l^{-1} min^{-1})	1.47 ± 0.27			1.35 ± 0.28			n.s.
VAS							
Satiety (mm VAS)	9 ± 2	66 ± 5	<0.001	10 ± 3	56 ± 6	<0.001	0.02
Hunger (mm VAS)	80 ± 5	33 ± 6	<0.001	79 ± 5	36 ± 6	<0.001	n.s.
Rewarding value							
Liking (average · $item^{-1}$)	68.03 ± 5.85	62.73 ± 7.49		63.01 ± 5.12	59.64 ± 7.33		n.s.
Wanting (items · $category^{-1}$)	0.93 ± 0.11	0.61 ± 0.10	<0.01	1.00 ± 0.12	0.67 ± 0.10	<0.02	n.s.
fMRI							
Right amygdala (AUC %BOLD s)	3.36 ± 1.99	1.97 ± 2.05		−9.97 ± 4.41	−7.34 ± 4.31		Condition <0.05
Cingulate cortex (AUC %BOLD s)	3.68 ± 0.37	3.03 ± 0.47		2.34 ± 0.70	1.78 ± 0.34		Condition <0.02
Hippocampus (AUC %BOLD s)	3.53 ± 1.36	1.76 ± 0.56		0.97 ± 0.74	−0.58 ± 0.78		Condition <0.02
Putamen (AUC %BOLD s)	2.69 ± 0.76	2.27 ± 0.75		1.98 ± 0.42	0.82 ± 0.37	<0.05	n.s.
Energy content							
Energy intake (MJ)	2.7 ± 0.3	1.6 ± 0.3	<0.05	2.7 ± 0.4	2.0 ± 0.4		n.s.
Energy density (MJ g^{-1})	10.4 ± 1.4	8.5 ± 1.6		10.6 ± 1.5	11.6 ± 1.5		<0.06
Food characteristics							
Crispiness (arbitrary units)	1.5 ± 0.2	1.4 ± 0.2		1.2 ± 0.1	1.2 ± 0.3		0.043
Creaminess (arbitrary units)	1.8 ± 0.2	1.6 ± 0.3		1.7 ± 0.2	1.5 ± 0.3		0.850
Fullness of taste (arbitrary units)	2.2 ± 0.2	2.0 ± 0.4		2.2 ± 0.2	2.2 ± 0.2		0.024
Sweet (arbitrary units)	1.7 ± 0.3	2.1 ± 0.6		1.7 ± 0.3	2.0 ± 0.3		0.737
Sour (arbitrary units)	1.6 ± 0.3	1.5 ± 0.3		1.5 ± 0.3	1.5 ± 0.3		0.981
Salty (arbitrary units)	1.8 ± 0.2	1.3 ± 0.3		1.7 ± 0.2	1.6 ± 0.3		0.055
Bitter (arbitrary units)	1.3 ± 0.1	1.2 ± 0.1		1.3 ± 0.1	1.3 ± 0.1		0.189
Macronutrient composision							
Carbohydrates (g)	85.6 ± 3.8	52.9 ± 3.5	<0.03	86.4 ± 3.8	59.0 ± 4.6		n.s.
Protein (g)	22.4 ± 1.1	7.8 ± 0.7	<0.001	22.8 ± 1.1	12.7 ± 1.1		n.s.
Fat (g)	21.9 ± 1.1	21.1 ± 1.1		22.1 ± 1.3	20.3 ± 0.8		n.s.

Abbreviations: AUC, area under the curve; BOLD, blood oxygen level dependent; ROI, region of interest; VAS, visual analog scale; Hormone data indicate the condition and were obtained five times per condition and are shown as one AUC (mmol l^{-1} min^{-1}) for each condition (rest and stress). All other data are given separately for the fasted and satiated state in the rest and the stress condition. All values are represented as average ± s.e.m. 100 mm VAS for hunger and satiety were given at five times. The results are shown as average for the fasted and the satiated state in both conditions. Data from predetermined ROI were extracted and are shown as AUC (mm VAS) for each state and condition. Energy intake (kJ), energy density (kJ g^{-1}), food characteristics (arbitrary units) and macronutrient composition (g) was determined for each selected meal and averaged over all subjects per meal in the respective condition.

To determine the effect of choosing food compared with choosing nothing, fMRI data were contrasted accordingly (Figure 2). There was significant activation in the left frontal cortex (possibly Brodmann area 10, $P<0.05$, FDR corrected).

To test the effects of eating breakfast on the representation of food choice-related brain activation, fMRI data were contrasted for choosing breakfast versus choosing the second meal. Significantly, lower activation was seen in the right putamen and the orbitofrontal cortex of satiated subjects (Figure 3, $P<0.05$ FDR corrected).

When comparing the stress condition with the rest condition during breakfast selection, lower activation was present in multiple brain areas: first, in the group contrasts, significantly lower activation was observed in the orbitofrontal cortex, frontal cortex and the putamen (Figure 4, FDR $P<0.05$). Second, ROI analysis of the Amygdala, the cingulate cortex and the hippocampus also showed lower activation in the stress condition compared with the rest condition (Table 2). Finally, the analysis of the putamen ROI revealed that in area of the whole putamen there was significantly reduced activation in the satiated stressed condition, compared with all the other conditions (Table 2).

Discussion

The main objective of the study was to determine the effect of stress on food choice and food reward. As a starting point,

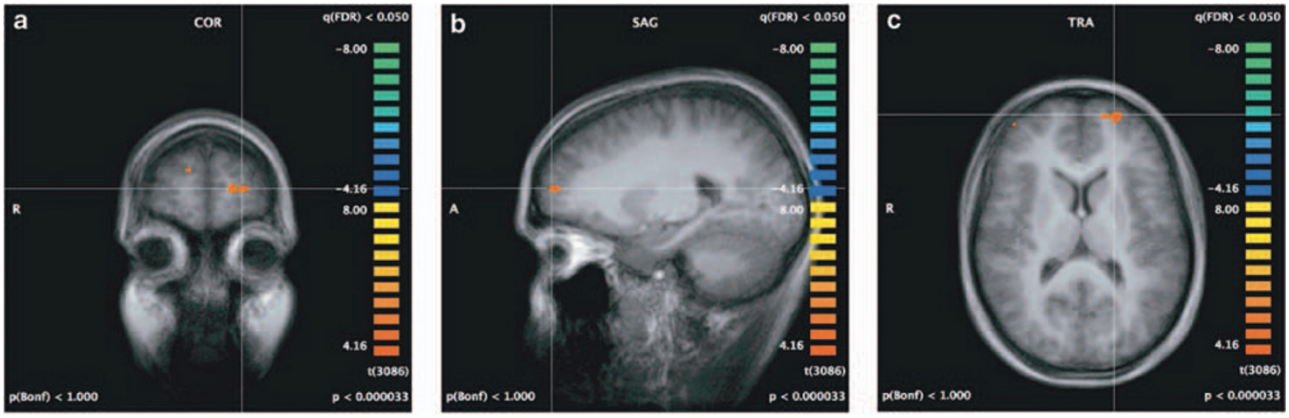

Figure 2 Sagittal (**a**), coronal (**b**) and transversal (**c**) sections, showing the GLM contrast of choosing something versus choosing nothing. Significant activation is visible in the left frontal cortex (−21, 58, 13; $P<0.05$ FDR corrected).

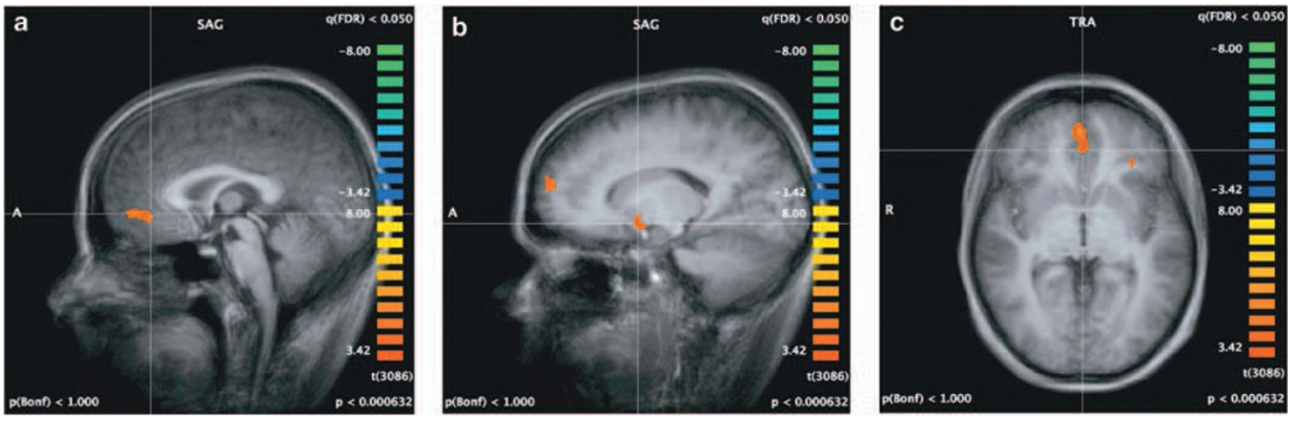

Figure 3 Two sagittal (**a**, **b**) sections at different planes and a transversal (**c**) section with the GLM contrast of choosing breakfast versus choosing a meal postprandially. There is a clear activation in the orbitofrontal cortex (3, 48, −1), frontal cortex (13, 61, 20 and −12, 63, 21) and putamen (18, 5, −5; $P<0.05$ FDR corrected).

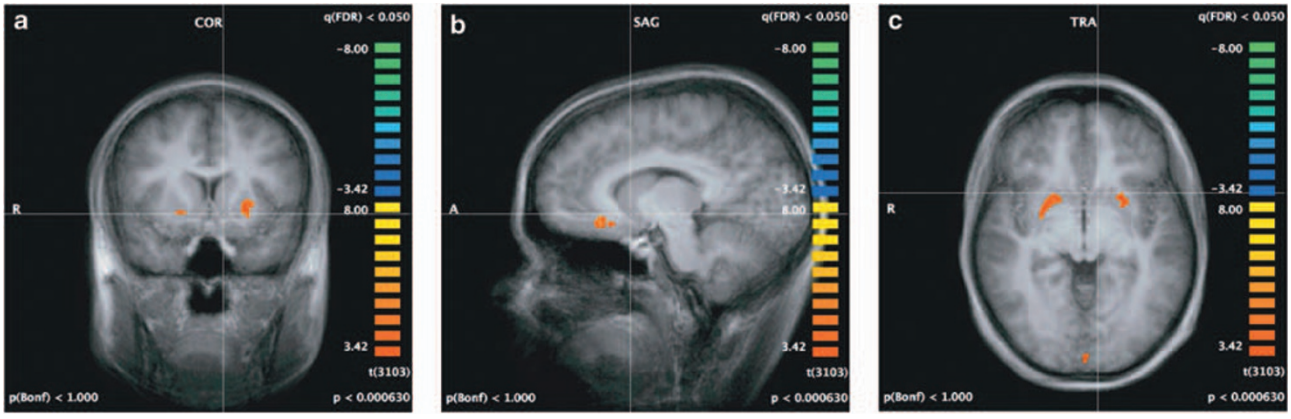

Figure 4 Sagittal (**a**), coronal (**b**) and transversal (**c**) sections, showing the GLM contrast rest condition versus stress condition. Significant activation in present in the putamen (22, 4, −3 and −23, 11, 4) and the orbitofrontal cortex (−8, 28, −6, $P<0.05$ FDR corrected).

only normal weight women were included. We confirmed that the method we used induced an endocrinological state of moderate acute stress, which was reflected by significantly elevated cortisol levels in the stress condition. As the effect of stress on food choice and food reward may be mediated by hunger or satiety, we executed the tests in a hunger as well as a satiated condition, both of which were also confirmed by relevant VAS scores.

In terms of the total food choice, energy intake was relatively higher postprandially in the stress condition compared with the rest condition. Additionally, subjects were similarly satiated postprandially in both conditions (Table 2, VAS hunger), whereas satiety was lower in the stress condition compared with the rest condition (Table 2, VAS satiety, interaction effect $P<0.02$). This is in line with previous findings on eating in the absence of hunger, where it was found that subjects under stress had a higher energy intake compared with subjects at rest.[23] Nevertheless, liking and wanting were not different under stress compared with rest: we found that the number of food items that were chosen was lower in the satiated condition compared with the fasted condition, whereas there was no difference between the stress and rest condition. The average relative liking of the food item was not different during stress compared with rest, and in the fasted condition compared with the satiated condition. Therefore, the total rewarding value of the food items, which were chosen and which are the combination of liking and wanting,[24] was lower as an effect of satiety but not as an effect of stress.

In contrast to the absent effect of stress on liking and wanting, we found that the food choice under stress compared with rest was different in terms of the food characteristics: subjects chose food items postprandially, which were higher in crispiness and fullness of taste in the stress condition compared with the rest condition. Consequently, the amount of carbohydrates and proteins in the selected food items was relatively higher during the second meal in the stress condition compared with the rest condition. This ultimately led to a trend toward choosing food items with higher energy density in the stress condition compared with the rest condition, resulting in eating in the absence of hunger. Overall, our findings support the idea that stress causes altered food choice, however, it does not lead to increased choice for carbohydrate and fat as proposed before,[15,17–20] but to increased carbohydrate and protein intake instead. We suggest that the likely cause for this difference is that our study population consisted of normal weight females, whereas the previous studies were conducted with obese and obesity prone subjects and the difference between the findings may, in fact, be a key component in the development of obesity. Surprisingly, our present findings indicate that the effects of stress are on food choice are clearly not only present in obese or obesity prone subjects. Furthermore, those effects are not limited to severe stress: our results show the effects of moderate stress that was indicated by significantly elevated cortisol but unaffected leptin and insulin levels.

With respect to relevant brain areas, whole brain contrasts were made and the resulting relevant active areas specific were defined as ROI from those. These regions included the putamen (dorsal striatum), amygdala, hippocampus and cingulate cortex, all of which have been shown to be involved in reward signaling.[25–30] Using GLM group contrasts on the fMRI data it was found that reward signaling-associated regions were significantly less active while choosing food in the satiated condition compared with the fasted condition (Figure 3) and while fasted in the stress condition compared with the rest condition (Figure 4), respectively. Using ROI event-related averages, significantly lower activation was seen in the amygdala, hippocampus and cingulate cortex in the stress condition, compared with the rest condition. Thus, stress seems to decrease the sensitivity of the reward system to food cues in general, which is reflected in decreased activation in food reward-associated areas.

The shift in food choice toward more crispiness, fullness of taste, and a tendency toward higher energy density in the stressed satiated condition compared with the stressed fasted condition, coincided with a lower activity of the putamen. As the putamen is also known as a movement-related pathway,[42] a contrast for choice for food versus choice for no food while viewing the images was included to confirm that putamen activation did not merely reflect extremity movement signaling (Figure 2). In this specific contrast, there was a significant activation in the left frontal lobe (possibly Brodmann area 10), which is reportedly involved in integration of different cognitive processes.[43] Therefore, the observed effects in the putamen were not because of movement encoding: if moving the joystick and pressing the button to choose something did not activate the putamen by itself, then the signal in the other contrasts could not have originated from this motion alone.

Using ROI event-related averaging analyses, we found lower activation in the putamen in the combined stress satiated condition. Considering previous evidence that the putamen is involved in reward signaling and reward prediction,[27,44,45] our data suggest that putamen activity may reflect a specific decrease in reward prediction sensitivity: choosing the different sized meals for breakfast and the postprandial second meal, lead similar putamen activation in the rest condition, indicating that the chosen items were predicted as a sufficient meal. In contrast, in the stress condition, the activation in this area was significantly lower during postprandial second meal selection, although the energy content of the selected second meal was not significantly different from the breakfast. Additionally, the striatum has been described to integrate activation from reward pathways and more behavior-related pathways.[46] Our data suggest that the putamen integrates the information of hunger and satiety with the predicted food reward to determine the predicted plausibility of the food choice. This prediction was altered in the stress condition, which lead to compensatory food choice toward items with higher energy content.

Overall, we found that stress interferes with the effects of hunger on energy intake, through lower satiety, a shift in food choice toward food that were higher in carbohydrates, proteins, crispiness and fullness of taste, and that had a tendency to higher energy density on average. This explained relatively higher energy intake in the postprandial

state compared with breakfast in this condition. It has been hypothesized that stress may affect food choice. We showed that stress decreased the activation in several reward-related brain areas, using GLM contrasts as well as ROI analyses, and that the putamen may play a complex integrating role in linking reward signals to behavior. Ultimately, stress caused higher energy intake, which was attributed to choice for foods with higher carbohydrate and protein content, different structural characteristics and most likely a difference in energy density.

Conflict of interest

The authors declare no conflict of interest.

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Author contribution

JMB conducted the experiment, including fMRI, developed the fMRI data analysis, analyzed the data and wrote the manuscript. SGTL conducted the experiment, assisted with fMRI and reviewed the manuscript and developed the liking and wanting paradigm. FR advised on the fMRI paradigm and reviewed the manuscript. AGN advised on the setup of the experiment concerning the acute stress paradigm and reviewed the manuscript. EF and RG advised on the setup of the fMRI paradigm, data analysis and reviewed the manuscript. MSWP conceived the experiment and developed the liking and wanting paradigm reviewed the manuscript.

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