Effects of mindset on hormonal responding, neural representations, subjective experience and intake

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ARTICLE INFO
Keywords:
Eating behaviour
fMRI
mesocorticolimbic system
ghrelin
glp-1
mindset
obesity

ABSTRACT
A person can alternate between food-related mindsets, which in turn may depend on one’s emotional state or situation. Being in a certain mindset can influence food-related thoughts, but interestingly it might also affect eating-related physiological responses. The current study investigates the influence of an induced ‘loss of control’ mindset as compared to an ‘in control’ mindset on hormonal, neural and behavioural responses to chocolate stimuli. Mindsets were induced by having female chocolate lovers view a short movie during two sessions in a within-subjects design. Neural responses to visual chocolate stimuli were measured using an ultra-high field (7T) scanner. Momentary ghrelin and glucagon-like peptide 1 (GLP-1) levels were determined on five moments and were simultaneously assessed with self-reports on perceptions of chocolate craving, hunger and feelings of control. Furthermore, chocolate intake was measured using a bogus chocolate taste test. It was hypothesized that the loss of control mindset would lead to hormonal, neural and behavioural responses that prepare for ongoing food intake, even after eating, while the control mindset would lead to responses reflecting satiety. Results show that neural activity in the mesocorticolimbic system was stronger for chocolate stimuli than for neutral stimuli and that ghrelin and GLP-1 levels responded to food intake, irrespective of mindset. Self-reported craving and actual chocolate intake were affected by mindset, in that cravings and intake were higher with a loss of control mindset than with a control mindset. Interestingly, these findings suggest that physiology on the one hand (hormonal and neural responses) and behavior and subjective experience (food intake and craving) on the other hand are not in sync, are not equally affected by mindset.

1. Introduction
In today’s society, more than 1.9 billion adult people are estimated to be overweight or obese [79]. The major cause of overweight is a prolonged energy imbalance, with the number of consumed calories exceeding the number of expended calories [25]. Though overweight people frequently attempt to lose weight, the number of successful weight loss maintainers is low [19]. Increased responding to food cues or contexts that signal the availability of tasty foods potentially sabotages healthy eating in dieters and weight loss maintainers [63]. This increased responding to food cues or food contexts includes increased food cravings, hunger related hormonal responses and reward-related...
neural activation; this so-called food cue reactivity prepares for intake and easily leads to overeating [5, 30, 32, 33]. Variance in food cue reactivity was found to account for 26% of the variance in eating and weight gain [5].

The increasing responding to food cues and contexts has been associated with specific mindsets (see e.g., [32, 66]). By priming someone’s thoughts, beliefs, assumptions and/or expectations, a certain mindset can be induced [61]. In the eating behaviour context, this mindset may for example be focused on health or enjoyment. It has been demonstrated decades ago that beliefs about caloric load can influence eating behaviour (see e.g., [78]). More recent studies investigating the effects of health claims or caloric content labels on high caloric food consumption support the early findings: Food intake is increased when a label indicates that the food is low caloric or healthy while intake is decreased when a label indicates that the food is high caloric content or unhealthy (e.g., [41, 58, 65]). Likewise, control and loss of control mindsets are associated with the inhibition and disinhibition of food intake [63]. Mindsets are assumed to be dynamic, meaning that a person can alternate between mindsets, depending on for example one’s emotional state or the situation [57]. Overweight people and unsuccessful dieters often report a ‘loss of control’ mindset; they frequently believe that they are unable to stop or prevent (over)eating when triggered by specific cues. Though the effects of labels on food intake have been studied a number of times, the effects of clinically relevant control versus loss of control mindsets have never been manipulated in experimental studies. Therefore, the current study aims to investigate whether and how an-induced ‘loss of control’ mindset as compared to an ‘in control’ mindset affects neural representations of visual chocolate stimuli, hormonal responses to the anticipation and consumption of chocolate, subjective experiences and the amount of chocolate consumed in females with a healthy weight.

1.2. Neural responses to food

While peripheral hormones interact with regulatory brain areas, prompting increase or decrease in hunger feelings [44, 48], eating behaviour is not solely a result of homeostatic hunger, but also a result of hedonic hunger related to the rewarding value of food [3, 11, 39]. To shed light on the neural response involved in processing the rewarding value of food, many functional magnetic resonance imaging (fMRI) studies have been conducted (e.g., see review [74]). However, the results on the rewarding value of food from studies with healthy-weight people (see [74]) and studies comparing healthy-weight to people who are overweight (see e.g., [81]) have been rather inconsistent. A reason for this inconsistency might be that mindset was not considered in these studies. That is, these studies often employed a so-called passive viewing paradigm, in which participants view food stimuli without specific instructions (e.g., [40, 59, 69, 72]). Importantly, in a passive viewing paradigm, it is unclear what the ongoing mental process of the participant is. While the researcher may assume that the participant is evaluating the tastiness or reward value of the food, the focus may just as well be on the healthiness or caloric content of the presented foods.

1.3. How mindset affects neural responses to food

Some fMRI studies have previously addressed how mindsets and task demands affect neural responses to food stimuli ([4,13, 21, 26, 27, 35, 61, 66, 75]). More specifically, it has been shown that neural responses in the mesocorticolimbic system were greater in overweight people than in healthy-weight people when participants were required to focus on the tastiness of stimuli, but not when they were not given any instructions on how to evaluate the presented foods [21]. Moreover, neural activity has been shown to be decreased by suppressing food craving related-thoughts while viewing high caloric food stimuli [66]. One of our recent studies likewise showed that neural activity in the mesocorticolimbic system was greater when the task demands required participants to evaluate the tastiness of the presented foods as compared to when they were required to evaluate the colors of the exact same foods in females who are overweight [22]. Another study showed that neural taste and pleasure experiences can be strongly influenced by people’s beliefs, as induced by label contents. Neural activity in the orbitofrontal cortex (OFC) while tasting wine, was purely influenced by manipulating wine price labels. While presenting the same wine stimuli, higher priced wines were rated as more pleasurable and the perceived pleasure correlated with increased activity in the OFC as compared to lower priced wines [52]. Likewise, brand labels presented with cola stimuli have also been found to affect taste ratings and neural reward signaling as represented in medial OFC, amygdala and striatum [38]. So, the dynamic nature of food-reward strongly depends on task demands, attentional focus and someone’s current mindset [57].

1.4. Does activity in the mesocorticolimbic system really reflect reward value?

There is a complicating factor in this line of research. While it is often assumed that neural activity in response to high caloric food stimuli in the mesocorticolimbic system is proportionate to the reward value of the presented stimuli, this is not at all straightforward. Instead, it has been proposed that activity in the mesocorticolimbic system reflects motivational saliency [60]. In support of this idea, it has been found that both rewarding and aversive stimuli led to neural activity in largely overlapping brain regions [8]. Moreover, in a previous study of our lab, we observed no significant differences in the level of neural activity in the mesocorticolimbic system between highly palatable high caloric and
highly unpalatable high caloric food stimuli [22]. Taken together, these findings suggest that activity in mesocorticolimbic brain areas reflect motivational saliency (either negative or positive), as opposed to reward value.

This raises the question how the brain codes the reward value if it is not reflected in the average level of activity in certain brain regions. It has been suggested that reward value might be reflected in so-called multivoxel patterns of neural activity in both the lateral and medial OFC [8, 71]. Cognitive regulation and food value-based choices were also found to be represented in neural multivoxel patterns of neural activity in the dorso-lateral prefrontal cortex (dIPFC, [73]). Dovetailing nicely with these findings, in a previous study from our lab, food palatability could be decoded from multivoxel patterns of neural activity, whereas we did not find significant food palatability differences using mass-univariate analyses [22]. Therefore, it is important to complement the standard mass-univariate analyses with Multi Voxel Pattern Analysis (MVPA), to better understand how the brain codes reward value, and how it might be affected by current mindset.

1.5. Study aim

To examine the influence of a general mindset on physiological mechanisms involved in appetite motivation, the current study measured the effects of a manipulated control and loss of control mindset in two separate sessions on hormonal, neural and behavioural responses to chocolate stimuli. In a within-subjects design, a loss of control and a control mindset were induced by having chocolate lovers view and engage in a short movie. Subsequently, neural responses to visual chocolate stimuli were measured using fMRI using a state-of-the-art ultra-high field (7T) scanner. Brain regions expected to be involved for chocolate craving were ventral striatum with nucleus accumbens (NAcc), midbrain and the OFC, whereas for control the inhibitory-control-related brain regions include: dIPFC and ventrolateral prefrontal cortex (vIPFC), parietal posterior cortex (PPC), dorsal anterior cingulate cortex (dACC), caudate, pre supplementary motor area (pre-SMA) and the globus pallidus (GP) [24]. To determine momentary active GLP-1 and ghrelin levels, blood samples were taken on 5 pre and post mindset induction moments and were simultaneously assessed with self-reports on perceptions of chocolate craving, hunger and feelings of control. Furthermore, chocolate intake was measured after the mindset manipulation using a bogus chocolate taste test. It was hypothesized that the manipulated loss of control mindset would lead to hormonal and neural responses that prepare for ongoing food intake, even after eating, while the manipulated control mindset would lead to hormonal and neural responses reflecting satiety. More specifically, a loss of control mindset – as compared to the in control mindset - was expected to lead to increased self-reported cravings and hunger, decreased feelings of control, increased chocolate consumption, increased ghrelin levels, and decreased GLP-1 levels. For the level of neural activity (mass-univariate analyses), we expected an increased activity in craving-related brain areas to chocolate versus neutral images in the loss of control mindset, whereas, more control-related activity was expected to chocolate stimuli in the control mindset. For multivariate analyses (using MVPA), differences were expected between the two mindsets in distinguishing neural representations of chocolate versus neutral images and predominantly in food-craving brain areas when in a loss of control mindset.

2. Material and methods

2.1. Participants

Twenty-six Dutch female undergraduates with a healthy weight were recruited by advertisements at Maastricht University. Inclusion criteria included: right-handiness, a female gender, a healthy weight (18.5 < Body Mass Index (BMI) < 25), liking of milk and dark chocolate (scoring on average ≥ 70 out of 100), having no MRI contra-indications and no history of neurological or psychological illnesses. Twenty-one participants used contraceptives (n= 17, birth-control pill; n = 4: hormonal IUD) and 17 females where tested in the luteal phase and 9 in the follicular phase of their menstrual cycle. This study was approved by the Medical Research Ethics Committee of Maastricht University Medical Centre. After completion, participants received € 100 in vouchers as compensation for their time. The sample size was based on a priori power analyses using G*power [18]. The inclusion of 26 participants in a within-subjects design provides sufficient power (> 0.9) with α = 0.05 for both hormonal (with an medium effect size of d = 0.25 based on [10]) and fMRI outcomes (with a large effect size of d = 0.8). The number of participants is also considered sufficient for a within-subjects fMRI design, based on simulation studies [9, 14, 45].

2.2. Experimental procedure

First, participants were screened for eligibility by filling in an online screening-questionnaire and when meeting the inclusion criteria, participants were scheduled for the two experimental sessions. In each session, one of the two mindsets was induced: (1) a loss of control mindset or (2) a control mindset. Order of conditions was counterbalanced across participants, so that 50% had first the loss of control mindset and 50% the control mindset. As the phase of the menstrual cycle may influence feelings of craving or neural responses to food [16], the two sessions took place at approximately the time of the menstrual cycle1. All sessions took place at the same time of the day (between 10:00-12:00 h AM). Participants were asked to arrive at the lab fasted (since bedtime, except for water), to control for hunger state differences and to assess baseline hormone levels from blood samples. Moreover, participants were asked to refrain from alcohol 24 hours before each session started.

Participants were informed about the whole experimental procedure and gave their informed consent. Then an intravenous canula was placed in an antecubital vein, and a fasted baseline blood sample (T1) was obtained. After 15 minutes, participants received a standardized low calorie breakfast (approx. 150 kcal), which consisted of a wholegrain cracker with cheese and a glass of orange juice (150 ml). After breakfast, a questionnaire on menstrual cycle and contraception was administered in the first session and the scanning procedure was explained. Fifteen minutes after breakfast, the second blood sample (T2) was obtained, which was followed by the one-hour scan-session. After the anatomical scan, the mindset induction took place in the scanner and directly afterwards, the third blood sample (T3) was taken. Then the acquisition of the 4 functional runs started. After the scan-session, the fourth blood sample (T4) was obtained and ad libitum chocolate intake was measured in a bogus chocolate taste test (see paragraph “Bogus chocolate taste test” below). Fifteen minutes after intake, the fifth blood sample (T5) was obtained. At each blood sampling time-point, participants indicated their chocolate craving, hunger and feelings of control on Visual Analogue Scales (VAS) (see Fig. 1 for timeline). At the end of the second session, height and weight were measured to calculate BMI.

2.3. Cognitive and Behavioural Assessments

2.3.1. Menstrual cycle and contraception

To have more insight in participants’ gonadal steroid hormone levels, participants were asked to fill in a short questionnaire on use of contraception and menstrual cycle.

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1 One participant had 2 months between 2 sessions, because of technical issues with the scanner during the 2nd appointment
2 One participant had chicken breast fillet instead of cheese, however the total calories consumed was the same
2.3.3. Chocolate liking

As part of the screening procedure, chocolate liking was measured with two questions on 100 mm Visual Analogue Scales (VAS) as part of the screening procedure: “How much do you like milk chocolate?” and “How much do you like dark chocolate?” ranging from 0 “not at all” to 100 “very much”. The average of the two rating outcomes was calculated.

2.3.4. Chocolate craving questionnaire

To assess participants’ trait chocolate craving, the Craving part (Factor 1) from the Attitudes to Chocolate Questionnaire (ACQ) [82] was administered at the end of the second session. This questionnaire consists of 17 items, individually scored on a Likert scale from -3 till 3 with a minimum score of -51 and maximum of 51. The internal consistency in the current sample was high, with a Cronbach’s alpha of 0.868.

2.3.5. Momentary self-reports

State chocolate cravings, hunger, and feelings of control over eating chocolate were assessed using 100 mm Visual Analogue Scales (VAS), ranging from 0 (not at all/ totally disagree) to 100 (very much/ totally agree). The questions included: “How much do you crave chocolate at this moment?” and “How hungry do you feel at this moment?” and were rated on the not at all – very much scale and the statement: “At this moment I would be able to control myself and not eat chocolate” was rated on the disagree – agree scale. The VASs were administered with paper and pencil, except for the one that was administered after the mindset manipulation in the scanner, for which a screen and joystick were used. Note that this digital measurement directly after manipulation could also be viewed as a manipulation check.

2.3.6. Bogus chocolate taste test

Ad libitum chocolate intake was measured in a bogus taste test after scanning in both sessions. During this taste test, participants rated milk chocolate and dark chocolate on taste, texture and liking using 0-100 mm VASs. The milk and dark chocolate (star-shaped pieces of ± 4 gram) were each presented in two cups with 20 grams each, so that participants were in total presented with 80 grams of chocolate (in total 437 kcal, 40 grams milk; 221 kcal and 40 grams dark: 216 kcal). The cups were labelled as A, B, C, and D, and presented as being slightly different. Participants were instructed to taste and rate the chocolate and it was announced that they could taste as much as they wanted. They were left alone during filling in the bogus taste test questionnaire. The bogus taste test had a set time of seven minutes, timed by the experimenter. After the taste test, the remaining chocolate was taken from the experimental room and weighed, to be able to compute the number of consumed calories. In the meantime, the participant filled in a questionnaire about her memory of the taste of the presented chocolates. This in-between questionnaire was administered to distract the participant during chocolate weighing and to maintain the cover story.

Because it was necessary for the measurement of ghrelin and GLP-1 that each participant had eaten an equal number of calories, the experimenter asked the participant to eat the remaining chocolate as well after the taste test. This requirement was disguised as a lottery, because otherwise participants would know that they would be asked to consume all chocolate in the second session as well. In this ‘lottery’, participants could draw a ticket, supposedly indicating ‘yes, eat all chocolate’, or ‘no, do not eat anymore chocolate’, whereas only ‘yes’- tickets were included. All participants ate the remaining chocolate after the taste test in both sessions.

2.4. Hormones

Blood samples were intravenously collected from an antecubital vein in ice-cooled, EDTA-coated tubes, with a total amount of 100 ccs (50 ccs per session) to determine active ghrelin and GLP-1 levels. Blood samples were directly centrifuged at 4°C, 3000 rpm for 10 min. and plasma was kept in a nitrogen bucket (± 200°C). Next, plasma was in duplo stored at -80°C until analysis. In the EDTA-coated tubes for GLP-1 analysis levels, the protease inhibitors dipeptidyl peptidase-IV (DPP-IV) (Millipore) was added to prevent degradation. Plasma for ghrelin determination was treated with methanol (HCL) and protease inhibitor phenyl-methyl-sulfonyl fluoride (PMSF) to prevent degradation. Ghrelin levels were measured using a double antibody radioimmunoassay (RIA) with an intra-assay variability of 15%. The lowest detectable active ghrelin was 26.11 pg/ml. Active plasma GLP-1 was determined by JJ Laboratory Services of University College London (UCL) with an assay sensitivity of 0.14 pmol/L, and with an intra-assay variability of 3 – 6 % and an inter-assay variability of 10 – 13 % by using commercial ELISAs (Cat. EZGLP-35K, Millipore).

2.5. Mindset manipulation

A mindset was induced using a short movie (± 75 sec), which participants viewed while in the MRI scanner prior to the acquisition of the functional runs. The movies consisted of pictures or short clips, with words and short sentences that were related either to ‘loss of control’ or to ‘control’ (see below for details). Participants were instructed to vividly imagine being in the situation as illustrated in the movie and to maintain the induced feelings as long as possible. After seeing the short movie, participants were asked to eat a small piece of chocolate (approx. 4 gr), to mimic the real-life situation with a control or loss of control.
feeling about eating something palatable and high caloric. To keep the mindset active during the functional scanning protocol, participants were primed eight times for three seconds during the fMRI task with 8 movie stills. The priming pictures were accompanied with the visual instruction: “Try to vividly imagine as much as possible being in the situation as previously shown in the movie.” The mindset induction movies (control and loss of control) were first tested in a between-subjects design including twenty participants (n = 10 for each condition) not taking part in the actual study. The results of this pilot study showed that the mindset induction movies had the intended effects: State craving was higher after the loss of control mindset induction (M = 69.50, SD = 14.58) than after the control mindset induction (M = 43.10, SD = 28.84, t(13.32) = 2.58, p < 0.05). Also, feelings of control (I want to control myself: t(11.84) = 5.53, p < 0.001, and I can control myself: t(18) = 3.16, p = 0.005) were stronger after the control mindset induction (want control: M = 80.00, SD = 7.87; can control: M = 80.70, SD = 1.57) than after the loss of control mindset induction (want control: M = 45.10, SD = 9.56; can control: M = 65.40 SD = 11.06).

2.5.1. Loss of control mindset
For inducing a loss of control mindset, pictures or short clips related to a hedonic lifestyle, foods, and enjoyable (social) situations were shown together with words/phrases to further emphasize the intended mindset. Example pictures/clips included: someone eating crisps, eating pizza with a group of people and a person relaxing on the beach with a nice cocktail. Example words/short sentences included: ‘enjoy’, ‘holiday’, and ‘let yourself go’.

2.5.2. Control mindset
For the control mindset, pictures or short clips consisted of healthy food items or healthy behaviour and being in control (social) situations. These pictures were also shown together with words/phrases to further emphasize the intended mindset. Example pictures/clips included: eating a healthy salad and a person standing on a scale, or doing some sports with others. Example words/short sentences included: ‘health’, ‘in control’, and ‘conscious’.

Both mindset videos were designed to be as similar as possible except for the crucial difference in eliciting control versus loss of control. Both mindset induction movies included social, food and non-food situations. A complete overview of included pictures/short clips and words/short sentences of both videos can be found in Supplementary Table 1.

2.5.3. Manipulation check
To check the perception of the induced mindset ‘control’ or ‘loss of control’, the participant was asked at the end of each session to what extent she could vividly imagine the depicted situations, and to what extent she could hold on to that feeling after seeing the mindset movie. Questions on feelings of being in control and chocolate craving after seeing the movie were also measured retrospectively at the end of each session using 100 mm VAS scales ranging from 0 “not at all” or to 100 “very much”. In addition, participants were asked to describe what they thought the aim of the study was to check if their ideas could have biased the results.

2.6. Visual stimuli presented during scanning
Stimuli included 40 pictures of chocolate (dark and milk) products and 40 neutral pictures of office supplies. Neutral pictures were not related to food and were matched as closely as possible on size to the chocolate pictures. Each picture was presented once per run and in total four times during the scanning session. Chocolate pictures did not have any festive associations (e.g., Valentine’s Day or Christmas). All stimuli were presented as pop-out figures on a light grey background (RGB: 191 191 191; CKYM: 25 20 20 0) in the center of a black screen, covering a visual angle of approximately 12°.

2.7. Stimulation protocol fMRI
The stimuli were presented in a blocked design using E-prime (version 2.0, Psychology Software Tools Inc., Pittsburg, PA, USA), with 2 block types of interest (chocolate and neutral stimuli) and 1 block type of no interest (question + mindset priming). Each block of interest lasted 16 s, and included either 8 chocolate or 8 neutral pictures. Each picture was selected randomly without replacement from the picture pool (until pool was empty), and presented for 2000 ms. To maintain the participant’s attention and to keep the mindset manipulation salient, two of the stimulus blocks were followed by a question + mindset priming block (3500 ms question, 6500 ms mindset priming). These question and mindset priming blocks were randomly placed in each run, with one occurring after a chocolate block and one after a neutral block. During these block types, first a picture was presented, and the participant was asked to indicate if that picture was also presented during the preceding stimulus block, by using a joystick. Next, the mindset priming was presented.

A fixation cross was presented before each block type (12 s for a stimulus block and 6 s for a question + mindset priming block) and after the last block (12 s). So, in total each run consisted of 5 chocolate blocks, 5 neutral blocks, and 2 question + mindset priming blocks. Each scanning session consisted of 4 runs, which in total lasted approximately 30 minutes. Prior to the functional runs, an anatomical scan (±10 minutes) was acquired.

2.8. MRI acquisition
All images were acquired on a 7T Siemens MRI scanner, equipped with 70mT/m, 200T/m/s whole body gradient coil and a 32 channel RF-coil. T1-weighted anatomical images and quantitative T1 maps were acquired using an MP2RAGE sequence with the following parameters: TR = 5000ms, TE = 2.47ms, TI1 = 900ms TI2 = 2750ms, flip angles = 5° and 3°, FOV = 224 × 196 mm and voxel size 0.7 × 0.7 × 0.7mm. Functional (T2*-weighted) images were acquired using a multiband gradient echo-planar imaging sequence (multiband factor 2, GRAPPA 3) with the Center for Magnetic Resonance Research (CMRR) multi-band C2P package [43, 64, 80], with the following parameters: TR = 2000 ms, TE = 18 ms, flip angle = 75°, FOV = 200 × 200 mm, and voxel size of 1.25 × 1.25 × 1.25mm. 92 axial slices were acquired in a backward tilted direction to the transversal – coronal line and covered the whole brain. Each participant underwent four experimental functional runs, with 172 volumes acquired per run. Thereafter, 5 functional images were acquired with opposite (anterior – posterior) phase encoding direction, for correcting magnetic susceptibility-induced distortions.

2.9. Preprocessing
Anatomical and functional data were preprocessed using BrainVoyager 20 (Brain Innovation, Maastricht, The Netherlands). Anatomical images were resampled to 1 × 1 × 1 mm resolution, followed by brain extraction, inhomogeneity correction and transformation to MNI space.

Functional data were corrected for slice scan time differences, and subjected to motion correction using 3 rotation and 3 translation axes. Low frequency drifts in the time course were removed using a temporal high pass filter, filtering out spatial frequencies below 3 cycles per run. Then, for each run a map of estimated magnetic susceptibility-induced distortions was estimated using the target volume and the volume with opposite phase encoding direction. This map was then used to correct all functional images for geometric distortions caused by non-zero off-resonance fields. Afterwards, data were spatially smoothed by using a 2 mm FWHM Gaussian kernel. After pre-processing, functional data were co-registered to the anatomical data and transformed to 4D MNI space.

As some participants’ motion resulted in significant spikes in their time courses, we submitted all functional data to a custom script that
searched to identify and replace affected volumes. A volume was deemed affected when 2.5 % or more brain voxels showed fluctuations in their time course of 4 standard deviations above or below mean signal. These volumes were replaced by a new volume, created by means of spline interpolation between the two adjacent volumes.

2.10. Cognitive, behavioural and hormone analyses

Self-reports of chocolate craving, hunger and feelings of control and blood hormone levels (ghrelin and GLP-1) were analyzed in 2 (condition: Loss of control vs Control) x 5 (time: T1 - T5) mixed ANOVAs. The difference in chocolate intake between both mindset conditions, as measured with the bogus taste test, was tested using a paired-samples t-test. Also, differences in the manipulation check outcomes, were analysed with paired-samples t-tests.

2.11. fMRI Analyses

2.11.1. Univariate analysis

Data were first submitted to a General Linear Model (GLM) in Brainvoyager, including the two factors (mindset: control / no control, and stimulus type: chocolate / neutral). In addition, for each participant, the question/priming blocks and the six motion parameters were added as confounds. Some volumes were quite noisy, which resulted in a stripping artefact. We have identified these affected volumes and used this information as a confounder as well. The GLM extracted the set of beta values per participant at each voxel (contrast images), which then served as input for the second-level random effects (RFX) analysis. We first tested the mindset x stimulus type interaction effect, and created a whole brain map with the omnibus F-statistic assigned to each voxel. Then, we tested and created a whole brain map for the main effects of mindset and stimulus type.

2.11.2. Multivariate analysis

Whereas mass-univariate analyses of fMRI data are informative regarding involvement of brain areas in certain tasks, multivariate analysis of fMRI data can decode representational content in the brain [49]. As this approach analyses multiple voxels at once, this could lead to a more sensitive detection of cognitive states. Therefore, we also used multi-voxel pattern analysis (MVPA) to test for differences in multivoxel representations of the two stimulus types between the two mindsets.

First, single trial responses were estimated by fitting a GLM to each voxel’s individual time course, thereby using one predictor for the trial of interest plus a second one that models all other trials simultaneously (see least squares simultaneous (LS-S) approach, [46]). Classification analyses were performed using functions of the CoSMo Multivariate Pattern Analysis (MVPA) toolbox [50] in Matlab. Data partitions consisted of classes of chocolate and neutral stimuli trials within a mindset (loss of control condition or control condition). Unequal trials between training and test partitions were balanced using CoSMo MVPA built-in functions [50]. We conducted a whole brain searchlight analysis [37], which is a descriptive method using a spherical ‘searchlight’ that centers on every voxel of the cortical volume, and gives an estimate of the presence of information in the spherical surrounding. On a single subject level, the algorithm targets all voxels individually and selects the voxels within the searchlight sphere with predefined radius of 6 mm surrounding the centred voxel. The algorithm then performs binary classification on the normalized single trial responses within the sphere’s voxels, using a linear Support-Vector (LIBSVM, https://www.csie.ntu.edu.tw/~cjlin/libsvm/). We trained and tested the classifier on discriminating between the two stimulus types (chocolate versus neutral). The classifier was trained on trial data of 3 runs and was tested on the trials of the remaining run, following a leave-one-run-out cross-validation procedure, resulting in 4 repetitions.

For group analysis, only voxels that showed total overlap across participants were included for further analyses, to exclude voxels with poor group overlap due to imperfect inter-subject brain alignment (i.e. voxels at/outside the cortex border). Mean accuracies of stimulus types within mindset were non-parametrically tested against chance level (0.5) and were FDR corrected on voxel-level. All mean accuracies significantly higher than chance were included for interpretation of the results. To test whether prediction accuracies differed between the two mindsets, the loss of control versus control mindset stimuli accuracy differences were non-parametrically tested against 0.

3. Results

3.1. Characteristics

Twenty-six healthy-weight female bachelor students were included (age: M = 20.31, SD = 1.52, BMI: M = 21.57 SD = 1.78). All participants were right-handed, had no MRI contraindications and scored high on chocolate liking (M = 86.42, SD = 9.66, range: 68 – 100). The average score on the Restrained Scale was M = 11.88 (SD = 4.82; range: 6.00 – 21.00). Overall participants had a relatively high score on the chocolate craving questionnaire (M: 9.11, SD: 13.40 with range: -20.00 – 38.00, of which 3 participants scored relatively low -15).

3.2. Manipulation check

At the end of each session, VASs were administered to test the effectiveness of the induced mindset manipulations. Participants reported a significantly stronger feeling of control after the control mindset induction (M = 73.57, SD = 16.65) as compared to the loss of control mindset induction (M = 51.84, SD = 21.79), t(25) = 5.69, p < 0.001, d = 1.12. Furthermore, chocolate craving was significantly lower for the control (M = 44.27, SD = 19.93) than for the loss of control condition (M = 80.58, SD = 13.91), t(25) = 9.14, p < 0.001, d = 1.79. Participants in both conditions scored high on vividness of the experience (control condition: M = 73.44, SD = 13.86, loss of control condition: M = 71.64, SD = 11.57), with no significant difference between conditions, t(24) = 0.61, p = .549, d = 0.12. The same was true for the degree to which they could hold on to the experience after the induction (control condition: M = 61.56, SD = 17.32, loss of control condition: M = 58.52, SD = 13.75), with no significant difference between conditions either, t(24) = 0.94, p = .359, d = 0.19. However, when comparing the two conditions on subjective ratings at T3 (directly after manipulation), only chocolate craving was significantly higher after loss of control as compared to control. Here, no significant differences were found on subjective feelings of control or hunger.

3.3. Cognitive and behavioural assessments

We tested for differences in hunger, chocolate cravings and feelings of control, between mindset conditions and across time in 2 (condition) x 5 (time) ANOVAs. No effects of mindset or mindset x time are observed for the momentary self-reports of hunger and feelings of control, all F < 4.06, all p > 0.057. This marginally significant effect of F = 4.06, p =0.057 is a main effect of mindset for hunger: hunger was marginally increased in the loss of control mindset. Main effects of time are observed for feelings of control, F(4,18) = 16.77, p < 0.001, Ï‰² = 0.79, and hunger, F(4,18) = 21.92, p < 0.001, Ï‰² = 0.83; feelings of control increase and hunger decreases over time. For chocolate craving, a main effect of time, F(4,18) = 32.38, p < 0.001, Ï‰² = 0.88, and a main effect of mindset, F(1,21) = 13.72, p = 0.001, Ï‰² = 0.39, are qualified by a marginally significant mindset x time interaction, F(4,18) = 2.44, p = 0.084, Ï‰² = 0.35. Chocolate cravings are overall higher in the loss of control mindset as compared to a control mindset and mostly at T3.

3 On vividness of the experience and the degree to which they could hold on to the experience, data of 1 participant is missing in 1 session
Fig. 2. State control, craving and hunger for the 5 timepoints (T1 – T5). Solid lines represent the Control condition, whereas dotted lines reflect the Loss of control condition. Error bars represent standard error of the mean. ** = p < 0.01 on paired t-test on T3 for craving. Abbreviation: VAS = Visual Analogue Scale, C = Control condition, LC = Loss of Control condition, B = Breakfast, M = Mindset manipulation, TT= taste test.

Fig. 3. Hormone levels of active grelin and GLP-1 on the 5 timepoints (T1 – T5). Solid lines represent the Control condition, whereas dotted lines reflect the Loss of control condition. Error bars represent standard error of the mean. Abbreviations: C = Control condition, LC = Loss of Control condition, B = Breakfast, M = Mindset manipulation, TT= taste test.
which is directly after the manipulation. The main effect of time seems mostly driven by a drop in craving between T4 and T5, which can be linked to the chocolate consumption. Fig. 2 gives the means and standard errors per time point per condition.

3.4. Bogus chocolate taste test

In line with our hypothesis, participants consumed significantly more kcal chocolate in the loss of control mindset condition (M = 182.81, SD = 68.30) than in the control mindset condition (M = 134.68, SD = 61.57), t(25) = 3.887, p = 0.001, d = 0.76.

3.5. Hormone levels

Of the ghrelin dataset 12 samples (6 in loss of control and 6 in control condition) were not included in the analysis, as these could not be accurately determined (inter-assay variability > 15 %). These missing data were imputed with the series mean of the specific timepoint within the condition. Statistical tests were performed with and without the imputed values and did not result in different interpretations of findings. Twenty participants were included for the GLP-1 analysis, because the last 6 participants’ GLP-1 values were unreliably determined due to a batch error. Ghrelin and GLP-1 both showed a main effect of time (ghrelin: F(4,22) = 9.22, p < 0.001, $\eta^2_p = 0.63$ and GLP-1: F(4,16) = 17.93, p < 0.001, $\eta^2_p = 0.82$). There was, however, no significant main effect of mindset (ghrelin: F(1,25) = 0.757, p = 0.393, $\eta^2_p = 0.03$ and GLP-1: F(1,19) = 1.93, p = 0.18, $\eta^2_p = 0.09$) and also the mindset x time interactions were not significant (ghrelin: F(4,22) = 0.48, p = 0.75, $\eta^2_p = 0.08$ and GLP-1: F(4,16) = 0.45, p = 0.77, $\eta^2_p = 0.10$). Hormone responding was generally in line with chocolate intake. The means per timepoint per condition are shown in Fig. 3.

4. Four measurements were missing, one per participant, for the repeated measures ANOVA and one for the t-test.

3.6. Whole-brain univariate analysis

We tested the main effects of mindset (control vs no control) and stimulus type (chocolate and neutral) and the mindset x stimulus type interaction wholebrain. The main effect of mindset and the mindset x stimulus type interaction were not significant (all voxels p > 0.05, FDR-corrected). A main effect of stimulus type (chocolate > neutral) was found, including brain regions of the mesocorticolimbic system, which is reported to be involved in craving and food-cue reactivity [24]: insula, OFC, amygdala, putamen, lateral occipital cortex (LOC) (p < 0.05, FDR corrected). See Table 1 and Fig. 4 for a more detailed report of significant clusters. Therefore, in several regions of the mesocorticolimbic system, the neural response for chocolate stimuli was larger than for neutral stimuli, and this effect was not moderated by the mindset induction.

Table 1.

3.7. Correlation neural activity with chocolate cravings

With post hoc correlation analyses, we tested whether trait chocolate craving was associated with neural activity, by entering the trait chocolate craving (ACQ) score as a covariate in our original GLM for univariate analysis with mindset (control vs no control) and stimulus type (chocolate and neutral). ACQ scores did not correlate significantly with the whole-brain main effect of stimulus type (all voxels p > 0.05, FDR-corrected). So, trait chocolate craving was not significantly related to neural responses to visual chocolate stimuli. We also tested post-hoc if a significant correlation between state chocolate craving and neural activity in response to food versus neutral stimuli would be observed right after the mindset induction. We entered the state craving scores at timepoint 3 (directly after both mindset inductions) as covariates for each mindset in our original GLM for univariate analysis. Again, no significant correlation between state chocolate craving and neural responses to food versus neutral stimuli was observed (all voxels p > 0.05, FDR-corrected).
Using a whole-brain searchlight approach [37], we tested if stimulus type (chocolate versus neutral) could be decoded above chance from patterns of neural activity by calculating classification accuracies, using a 6 mm radius sphere, per voxel. We expected that classification of stimulus type (chocolate versus neutral) would be more accurate for the loss of control mindset in the mesocorticolimbic system, whereas we expected classification to be more accurate for the control mindset in control-related brain areas.

Within both mindsets, stimulus type could be decoded significantly above chance per condition, but decoding accuracy did not differ significantly between conditions.

### 3.8. Whole-brain multivariate analysis

Using a whole-brain searchlight approach [37], we tested if stimulus type (chocolate versus neutral) could be decoded above chance from patterns of neural activity by calculating classification accuracies, using a 6 mm radius sphere, per voxel. We expected that classification of stimulus type (chocolate versus neutral) would be more accurate for the loss of control mindset in the mesocorticolimbic system, whereas we expected classification to be more accurate for the control mindset in control-related brain areas.

Within both mindsets, stimulus type could be decoded significantly above chance (voxel-wise FDR corrected). The clusters largely overlapped with the results of the mass-univariate analysis of the main effect of stimulus type (Fig. 5), including areas in the bilateral (dorsal and ventro-lateral) prefrontal cortices, insulae, amygdalae, parietal cortices and lateral occipital cortex (LOC).

We subsequently tested whether this decoding accuracy differed between mindset conditions. However, no clusters had a significantly higher decoding accuracy in the loss of control as compared to the control mindset condition or in the opposite direction after voxel-wise FDR correction. So, stimulus type could be significantly decoded above chance per condition, but decoding accuracy did not differ significantly between conditions.

### 4. Discussion

The aim of this study was to examine the influence of a loss of control versus a control mindset on hormonal and neural responses, self-reported cravings, hunger, control and chocolate intake. The current study shows that (1) neural activity in several regions of the mesocorticolimbic system is stronger for chocolate stimuli than for neutral stimuli, and this effect is not moderated by mindset, (2) ghrelin and GLP-1 levels respond to food intake, but they are not affected by mindset, and (3) self-reported craving and actual chocolate intake are affected by mindset, in that cravings and intake are higher with a loss of control mindset than with a control mindset. Interestingly, our findings show that subjective experience and behaviour are influenced, whereas hormonal and neural responses are not influenced by the induced mindset. In addition, our findings suggest that physiology (hormonal and neural responses) are not in sync with behaviour and subjective experiences, as they are not equally affected by mindset.

The predicted mindset effect observed for momentary craving and...
chocolate intake, is in line with previous studies investigating cognitive modulations of eating behaviour, showing that beliefs generated by health claims or caloric content labels of high caloric food products influence intake (e.g., [41, 58, 65]). This is also in line with previous research that showed that a health mindset attenuates attention towards high caloric foods [77]. Recent studies also showed that food cravings and food valuation are affected by cognitive strategies, like regulation or reappraisal of food cravings [6, 55]. In contrast with these previous studies, our manipulation was not directed at the food product itself (e.g., with caloric content labels), instead we aimed to induce a more general state of mind of “letting yourself go” in the loss of control mindset versus “being in control” in the control mindset. Our data fit with cognitive models of overeating and binge eating, in that beliefs are determinants of behavior. This means that eating behavior does not occur ‘in a vacuum’, but is affected by beliefs and thoughts (see e.g., [2, 31]).

In this study, we did not find any significant top-down neural control-related responses after inducing a control mindset, nor any indications for stronger neural craving-related responses by inducing a loss of control mindset. A possible explanation for this unexpected effect, is that all participants scored high on trait chocolate craving. Seeing chocolate stimuli might have triggered neural activity in the mesocorticolimbic system, independent of the induced mindset [24]. The automatic response might be stronger for these high trait chocolate cravers than a quite subtly induced mindset. There is indeed some evidence [42] that high trait chocolate cravers show more implicit pleasure related neural responses in the mesocorticolimbic system towards chocolate pictures as compared to low chocolate cravers, without strict instructions during viewing.

Interestingly, previous research showed when chocolate cravers were explicitly instructed to suppress their chocolate craving thoughts while viewing chocolate pictures, both the craving and activity in the mesocorticolimbic system were reduced [42]. This finding converges nicely with recent work from our laboratory, showing that an explicitly induced attentional focus (i.e., 1-back task with focus on taste versus colors) while viewing food images, strongly influenced participants’ neural responses to high-caloric food pictures [22]. That is, the neural response in the mesocorticolimbic system was significantly higher with a hedonic than with a neutral focus, independent of the palatability of the presented food items. Because in the current study the mindset induction was not as explicit, that is not directly directed at chocolate craving, the resulting mindset might not have been strong enough to elicit conflict in neural responding to chocolate stimuli [15, 29]. So, a second explanation for not finding the predicted effects in the current study, could be that the mindset induction was not as explicit as it was not part of task requirements (as with our 1-back task in [22]) and did not induce an internal conflict during neural chocolate processing.

Third, some studies indicate a difference between anticipatory and consummatory food reward processing [67, 68]. Whereas anticipatory food reward entails the anticipated rewarding value of a food cue, consummatory food reward is the experienced rewarding value after actual intake. It could be that this type of induced mindsets would interfere with neural responses during scanning when participants also correspondingly receive actual chocolate (or chocolate milk). Previous research also showed that eating behaviour and self-control differs between actionable food temptations (directly accessible food) or non-actionable food temptations (not directly accessible foods, e.g., pictures of food) [23]. So, our control / loss of control mindsets might have affected consummatory reward instead of anticipatory rewards, as we did find differences in actual eating behavior (i.e., chocolate consumption during the bogus taste test).

As expected, independent of mindset, we did find increased neural
activity to chocolate as compared to neutral stimuli in brain regions that have previously been reported to be involved in food-craving or food-cue reactivity (e.g., insula, OFC, amygdala, putamen, lateral occipital cortex (LOC)) [24, 53, 71, 74]. This is different compared to our previous results, where we did not find any neural differences between food stimuli [22]. However, an important big difference to the previous study is the visual or motivational saliency of the stimuli presented. The food stimuli used in the previous study were equally salient to the participants (individually tailored highly palatable and highly unpalatable foods) as confirmed by not finding any differences in univariate neural responses earlier [8]. In the current study, chocolate pictures are likely being processed as more salient than the presented non-food office supplies stimuli, and this saliency seem to be even more pronounced for this specific group of high chocolate cravers. Our univariate neural findings therefore suggest that the saliency of chocolate stimuli overrules the possible effect of the induced – and less explicitly active and stimuli-engaged – loss of control or control mindset.

Whereas mass univariate analyses inform on involvement of activated brain areas, multivariate analyses inform on the representational content of these brain areas [49]. Though the decoding accuracy of chocolate versus neutral stimuli was significantly above chance, decoding accuracies did not differ significantly between mindsets. Decoding of chocolate versus neutral stimuli is in line with prior research, which showed that the subjective value of food stimuli could be decoded from multi-voxel patterns of neural activity [8, 71]. The multivariate analyses suggest that the subjective value of chocolate stimuli was not altered by the induced mindsets while processing the chocolate stimuli. In our previous study [22], we did observe that decoding accuracy of highly palatable versus highly unpalatable stimuli was significantly better for the hedonic focus than for the neutral focus. Again, the explanation could be that in the previous study the induction of mindset was very explicit and task-based (i.e., 1-back task with a focus on taste versus color).

Notably, effects on hormonal responding were unexpected as well; no significant differences were found in ghrelin and GLP-1 responses between the two induced mindsets. Hormones responded as expected over time and in response to eating moments, where ghrelin levels decreased, and GLP-1 levels increased after eating moments. The findings on anticipated hormone levels are however not in line with previous studies, finding expectations or beliefs to influence hormonal responding to food [7, 10]. A difference with the current study is that in the studies of Cassady et al. [7] and Crum et al., [10] expectations were directly linked to a specific food or drink, by attached labels, brands or product-specific caloric density expectations. We induced a more general mindset, and we did not induce any expectations about the satiating effects or caloric load of the eaten chocolate. Whereas higher chocolate craving was reported after the loss of control mindset, this was not accompanied by self-reported hunger or hormone levels. This might suggest that priming participants’ mindset or beliefs about specifically caloric content or satiating values of foods or drinks is needed to change hunger feelings and the accompanied hormone release.

Taken together, the current study gives some interesting leads for further investigation. This study illustrates that control and loss of control mindsets influence chocolate cravings and chocolate intake, whereas neural and hormonal responses are not influenced by the induction of these specific mindsets. One of the strengths of our study is the multidisciplinary set up, including several psychological and physiological measures. An ultra-high field (7T) MRI scanner was used to acquire fMRI data, which made it possible to examine neural responses in great detail. Moreover, fMRI data were analyzed using both mass-univariate and multivariate analyses. Participants served as their own controls, which made it a strong within-subjects design, as the experimental procedure was kept exactly the same in both sessions, except for the mindset induction. The group of participants was homogeneous, consisting of female chocolate cravers, thereby reducing variability. The disadvantage is that the results of the current study are less generalizable to other food cravings and to other populations (e.g., males). Another disadvantage to address is that it was not possible to examine neural responding to consummatory reward of accessible chocolate as this would lead to excessive movement in the scanner. Though further research is needed to confirm and expand these findings, the current study shows that momentary chocolate cravings and intake can be manipulated by a quite subtle mindset induction.

Author Contributions

S.F., Anne R. T.A. and A.J. designed the study. S.F. collected the data. S.F. and J.H. analyzed the data. The scanning protocol was set up by Alard R. S.F. and Anne R. wrote the manuscript, T.A., A.J., Alard R., K.G., and J.H. gave feedback on the manuscripts, and all authors approved the final version.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgements

This study was financed by the Netherlands Organization for Scientific Research (NWO), FBC-grant (057-13-010) and the Maastricht University Interfaculty Program ‘Eatwell’, both awarded to Anita Jansen. The authors would like to acknowledge Kyra Wijnen and Vincent van de Vlasakker for their assistance in data collection.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2022.113746.

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