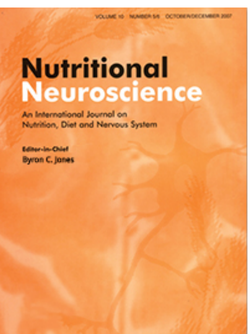

Nutritional Neuroscience

An International Journal on Nutrition, Diet and Nervous System

ISSN: 1028-415X (Print) 1476-8305 (Online) Journal homepage: https://www.tandfonline.com/loi/ynns20

# Brain activity and connectivity changes in response to nutritive natural sugars, non-nutritive natural sugar replacements and artificial sweeteners

Anna M. Van Opstal, Anne Hafkemeijer, Annette A. van den Berg-Huysmans, Marco Hoeksma, Theo. P. J. Mulder, Hanno Pijl, Serge A. R. B. Rombouts & Jeroen van der Grond

**To cite this article:** Anna M. Van Opstal, Anne Hafkemeijer, Annette A. van den Berg-Huysmans, Marco Hoeksma, Theo. P. J. Mulder, Hanno Pijl, Serge A. R. B. Rombouts & Jeroen van der Grond (2019): Brain activity and connectivity changes in response to nutritive natural sugars, non-nutritive natural sugar replacements and artificial sweeteners, Nutritional Neuroscience, DOI: 10.1080/1028415X.2019.1639306

**To link to this article:** https://doi.org/10.1080/1028415X.2019.1639306

© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group

Published online: 10 Jul 2019.

Submit your article to this journal

Article views: 2941

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=ynns20

NUTRITIONAL NEUROSCIENCE
https://doi.org/10.1080/1028415X.2019.1639306

# Brain activity and connectivity changes in response to nutritive natural sugars, non-nutritive natural sugar replacements and artificial sweeteners

Anna M. Van Opstal[a], Anne Hafkemeijer[a,c,e], Annette A. van den Berg-Huysmans[a], Marco Hoeksma [b], Theo. P. J. Mulder [b], Hanno Pijl[d], Serge A. R. B. Rombouts[a,c,e] and Jeroen van der Grond[a]

[a]Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands; [b]Unilever Research & Development, Vlaardingen, The Netherlands; [c]Department of Methodology and Statistics, Institute of Psychology, Leiden University, Leiden, The Netherlands; [d]Department of Internal Medicine, Leiden University Medical Center, Section Endocrinology, Leiden, The Netherlands; [e]Leiden Institute for Brain and Cognition (LIBC), Leiden, The Netherlands

**ABSTRACT**

**Introduction:** The brain plays an important regulatory role in directing energy homeostasis and eating behavior. The increased ingestion of sugars and sweeteners over the last decades makes investigating the effects of these substances on the regulatory function of the brain of particular interest. We investigated whole brain functional response to the ingestion of nutrient shakes sweetened with either the nutritive natural sugars glucose and fructose, the low- nutritive natural sugar replacement allulose or the non-nutritive artificial sweetener sucralose.
**Methods:** Twenty healthy, normal weight, adult males underwent functional MRI on four separate visits. In a double-blind randomized study setup, participants received shakes sweetened with glucose, fructose, allulose or sucralose. Resting state functional MRI was performed before and after ingestion. Changes in Blood Oxygen Level Dependent (BOLD) signal, functional network connectivity and voxel based connectivity by Eigenvector Centrality Mapping (ECM) were measured.
**Results:** Glucose and fructose led to significant decreased BOLD signal in the cingulate cortex, insula and the basal ganglia. Glucose led to a significant increase in eigen vector centrality throughout the brain and a significant decrease in eigen vector centrality in the midbrain. Sucralose and allulose had no effect on BOLD signal or network connectivity but sucralose did lead to a significant increase in eigen vector centrality values in the cingulate cortex, central gyri and temporal lobe.
**Discussion:** Taken together our findings show that even in a shake containing fat and protein, the type of sweetener can affect brain responses and might thus affect reward and satiety responses and feeding behavior. The sweet taste without the corresponding energy content of the non-nutritive sweeteners appeared to have only small effects on the brain. Indicating that the while ingestion of nutritive sugars could have a strong effect on feeding behavior, both in a satiety aspect as well as rewarding aspects, non-nutritive sweeteners appear to not have these effects.
**Trial registration:** This study is registered at clinicaltrials.gov under number NCT02745730.

**KEYWORDS**
MRI; energy ingestion; brain activity; eigen vector centrality; functional network connectivity; nutritive sweeteners; non-nutritive sweeteners; artificial sweeteners

## Introduction

The brain plays an important regulatory role in directing energy homeostasis and eating behavior and is an important area of interest for research in to the maintenance of a healthy energy balance [1]. Energy consumption is not only regulated by homeostatic processes, it also has hedonic aspects in which the brain has an important role [2,3]. Fats, proteins and carbohydrates, all have specific effects on the brain, and ingestion can elicit various functional brain responses [4,5]. The increased ingestion of sugars and sweeteners over the last decades increases the particular interest of investigating the effects of these substances [6–9]. Understanding of the functional brain responses to sugars or sweeteners could give insight into the effects of these common dietary substances on satiety signaling and feeding behavior involved in maintaining energy homeostasis.

Glucose is a commonly consumed natural sugar. It is the primary source of energy for the brain and its metabolism is kept under tight regulation. The brain consumes about 20% of glucose derived energy in the human body [10]. The brain responds readily to ingestion of glucose because of its quick absorption [10–12], and glucose sensing neurons in the hypothalamus show a homeostatic

**CONTACT** A. M. Van Opstal a.m.van_opstal@lumc.nl Department of Radiology, Leiden University Medical Center, Albinusdreef 2, Leiden 2300RC, The Netherlands

© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

satiety response almost immediately after ingestion [13–17]. Glucose ingestion has effects on both neuronal activity and functional connectivity throughout the brain in areas involved in reward and feeding behavior [16]. Additionally, circulating blood glucose levels influence brain responses and activity and the CNS regulation of glucose homeostasis [18–21]. This indicates that glucose consumption can have strong and widespread effects on the brain, satiety signaling and feeding behavior. However, many foods and beverages contain other mono- and di-saccharides, such as fructose, sucrose. More and more, low or non-nutritive sweeteners are used to sweeten foods and beverages [6,22]. The metabolic pathways of these sweeteners are all different. For instance; in contrast to glucose, fructose cannot be used directly as a source of energy but first has to be metabolized by the liver before it can be utilized by the brain [23–25]. The metabolic effects of non-caloric sweeteners are not straightforward. Non-caloric sweeteners are expected to decrease caloric intake and might therefore be useable to control obesity [26]. However, some epidemiological studies have found that non-nutritive sweeteners might actually increase energy intake [9,27]. Although several studies also show there is no association between sweeteners and increases in energy intake [26,28], indicating that the effects of the substances are not clear-cut. Earlier findings indicate that the homeostatic and hedonic responses, as measured by BOLD signal changes, by the hypothalamus and ventral tegmental area (VTA) are driven by sweet taste coupled with caloric content. Sweet taste without caloric content, as found in artificial non-nutritive sweeteners, does not seem to elicit a lasting response from these brain areas in this study [17]. Recently, allulose, a novel low-nutritive natural sugar replacement, has become available and is now used to sweeten various health products such as protein bars. Allulose, or D-psicose, is a very low-energy monosaccharide sugar that is derived from fructose [29]. Unlike sucralose and other artificial sweeteners, allulose is very similar in molecular structure, taste and texture to fructose and glucose. Allulose has a similar relative sweetness compared to glucose but contains only 0.2 kcal/g instead of 4 kcal/g. Additionally, allulose is transported from the gut lumen to the blood via glucose transporters, which has been shown to elicit reward signals [30]. Small doses of allulose have been shown to reduce the postprandial blood glucose response and seems therefore to be able to influence glucose homeostasis [31,32]. Because these types of sweeteners seem to influence glucose metabolism and are more similar in molecular structure and metabolism to natural sugars than other artificial sweeteners they could elicit brain response more similar to natural sugars.

Although, it is obvious that regulation and sensing of food in the brain is complex, with many parts of the brain involved, up to now functional MRI studies have mainly focussed on single areas, mostly hypothalamus, to study the effect of sugars and sweeteners. However, because of the complexity and involvement of various brain areas in the regulation of energy balance [33], fMRI investigations of whole brain responses might be more informative for this regulation. Measurements of local BOLD changes, a measure of neuronal activity [34], can be used to analyse the immediate effects of nutrient ingestion on very specific brain areas [13–15,35,36], but can also be used to determine whole brain effects on a voxel based level [37]. Analysis of functional connectivity has been used to provide further insights on the network level [16,38]. In this respect, various functional networks are involved in feeding behavior and energy balance. Networks of special interest are the default mode, salience and executive control network. The default mode network reflects a baseline state of the brain and has been shown to be altered/involved in a disrupted energy balance in obesity [39,40]. The salience network has been shown to be involved in feeding behavior and reward [41,42]. The executive control network is involved in decision making and impulse control and is important in maintaining energy balance [42,43]. A newer method to determine functional brain connectivity is eigenvector centrality mapping (ECM). ECM is used to determine the level and quality of connectivity on a voxel-wise level rather than on a network level [44,45]. Eigenvector centrality values have been shown to be correlated with states of hunger and satiety [44].

In the present study, we aim to investigate the effects of the ingestion of sweetened nutrient shakes containing fats and protein. These shakes were sweetened with either the nutritive natural sugars glucose and fructose, the low- nutritive natural sugar allulose, or the non-nutritive artificial sweetener sucralose, on whole brain neuronal activity and (network) connectivity. Our overall hypothesis is that while the natural sugars glucose and fructose elicit a response from the brain, the non-nutritive sweeteners might lack a similar response due to the lack of caloric content.

## Methods

### *Subject characteristics*

Twenty non-smoking, Caucasian men, aged 18–25 years were recruited through local advertising. Exclusion criteria were: a history of disturbances in glucose metabolism (e.g. diabetes mellitus), any significant chronic

disease, psychiatric disease, BMI below 20 or above 23 kg/m$^2$, body weight below 70 kg, body height below 170 or above 190 cm, recent weight changes (>3 kg gain or loss) within the last 3 months, having smoked within the last 6 months, recent blood donation, alcohol consumption of more than 21 units per week, recent use of recreational drugs, and contra-indications to MRI scanning. The study was approved by the local Medical Ethical Committee (NL 55440.058.15) and prospectively registered at clinicaltrials.gov (NCT02745730). All volunteers gave written informed consent before participation.

## Study design

The entire study was performed in a double blinded, 4-times crossover design. Resting state functional MRI (rsfMRI) was performed before and after ingestion of the four different conditions in a randomized sequence, using a batch wise randomization procedure, based on a Williams design balanced for first-order carry-over. The randomization was performed by an independent statistician who was not involved in the analysis of the study data. There was a wash out period of at least one week between each of the four study visits. On all occasions, rsfMRI was performed before and after ingestion of one of the following study conditions; glucose (23 gram of carbohydrate, 93 kcal), fructose (23 gram), sucralose (0.05 gram); matched for sweetness with glucose, or allulose (23 gram); matched for sweetness with glucose. All sugars were dissolved in 165 ml of a sugar free 'milkshake' consisting of water, sodium casseinate 0.20 wt % (0.33 grams of protein, 1 kcal) of protein. coconut oil 3.0 wt % (5 grams of fat, 45 kcal) and guar 0.40 wt %. Cocoa powder was added as flavoring. The dosage of the glucose and fructose (and also of the other macronutrients) is similar to the amount found in commercially available milkshake in fast food chains (per 100 gram of product). The dosage of the sweeteners was matched to glucose to insure that possible differences in brain response were not caused by differences in perception of sweet taste. The milkshake solution was made by Unilever Research & Development Vlaardingen B.V., The Netherlands, and was stored frozen. A full day before examination the frozen shakes were stored in a food-grade-refrigerator with a temperature of 4°C to defrost. All shakes were consumed at 4°C. Subjects were asked to fast overnight, i.e. after 10PM no food or drinks were allowed; only water. Drinking of the shakes was performed in the MRI room, sitting in an upright position on the MRI scanner table, outside the magnet bore. Subjects were instructed to taste the shake first with a few nips, then to drink the total amount in a steady and continuous way within 2 min. RsfMRI acquisition was continued 15 min after ingestion. Schematic representation and timing of the total fMRI study procedure is shown in Figure 1.

Prior to the study, we performed power analyses based on earlier findings showing a 1.9% change in BOLD after the ingestion of glucose [17]. In that same study, we found a 1.2% BOLD change after fructose ingestion, indicating that the BOLD effect between treatments is much smaller than the BOLD effect between pre and post-ingestion. In this respect, the current study is not sufficiently powered to draw reliable conclusions when comparing treatments directly. Still, the current 4-fold cross over design ensures homogeneity of the study groups across treatments.

## Visual analogue score analysis

Subjective feelings of hunger, fullness and wanting a meal at baseline and wanting to continue ingestion after first tasting and full ingestion of the shakes were indicated on a Visual Analogue Scale (VAS) which consisted of a 10 cm line, with 'not at all' and 'extremely' as anchors. Subjects were asked to indicate their score on the line, higher scores indicating a stronger feeling of hunger/fullness, etc. Differences in VAS scores between conditions were analyzed using a repeated measure ANOVA per VAS score using the Statistical Package of Social Sciences (SPSS, version 23.0; SPSS, Chicago, Ill). Correlations between VAS scores and quantitative functional MRI measures are calculated using Pearson correlations.

## MRI data acquisition

MRI scanning was performed on a Philips Achieva 3.0 T scanner using a 32-channel SENSE head coil (Philips

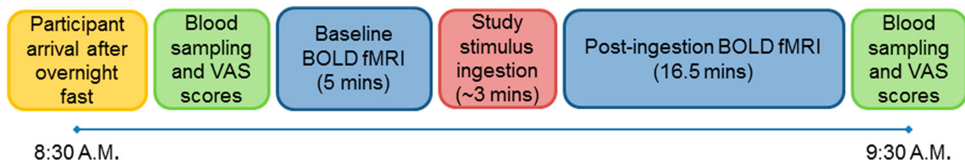


**Figure 1.** Schematic representation of the fMRI procedure.

Healthcare, Best, The Netherlands). Anatomical high-resolution 3DT1- weighted images of the whole brain were acquired (TR 9.8 ms, TE 4.6 ms, flip angle 8, 140 transverse slices, FOV 224 mm × 177 mm × 168 mm, reconstructed in-plane resolution 0.88 mm × 0.87 mm, slice thickness 1.2 mm) along with a high-resolution T2*-weighted EPI scan (EPI factor 35, TR 2200 ms, TE 30 ms, flip angle 80, 84 axial slices, FOV 220 mm × 220 mm, in-plane resolution 1.96 mm × 1.96 mm, slice thickness 2.0 mm) for registration purposes. Resting-state scans were acquired with T2*-weighted gradient echo-planar imaging (EPI factor 35, 160 dynamics, 37 transverse slices scanned in ascending order, TR 2200 ms, TE 30 ms, flip angle 80, FOV 220 mm × 220 mm, voxel size 2.75 × 2.75 × 2.50 mm with a 0.25 mm slice gap, total acquisition time: 6 min).

### MRI data preprocessing

MRI data were preprocessed and analyzed using Functional Magnetic Resonance Imaging of the Brain Software Library (FSL) [46], Matlab and Python. Of all data sets, structural and functional, non-brain structures were removed using the Brain Extraction Tool (BET) tool as implemented in FSL. The T1-weighted images were registered to the 2 mm isotropic MNI-152 standard space image using non-linear registration with a warp resolution of 10 mm. The FMRI Expert Analysis Tool (FEAT) was used for motion correction with MCFLIRT, spatial smoothing with a full width at half maximum of 3 mm, and high pass temporal filtering with a cut-off frequency of 0.01 Hz. The functional resting state images were registered non-linearly to the corresponding T1-weighted images using Boundary-Based Registration (BBR) affine registration, using the high-resolution echo planar images as an additional registration step.

### MRI data analysis whole brain BOLD changes

Whole brain voxel based BOLD intensities were compared before and after ingestion according to Rombouts et al [37]. In short, a single volume BOLD signal map was calculated by averaging the time series data. To decreases the possibility of including an unwanted signal of other compartments than CSF an average CSF signal of the single volume BOLD signal map was determined by averaging all voxels within the masked CSF. This CSF mask was determined by selecting voxels located in the lateral ventricles on the segmented structural images. Next, in each subject, a normalized BOLD signal map was calculated by dividing each voxel's signal by the average CSF signal. Voxel-wise comparisons of pre- and post-ingestion normalized BOLD signal maps were done using the Randomize tool of FSL with a paired samples approach and using Threshold-Free Cluster Enhancement (TFCE) [47]. All data were family wise error (FWE) corrected at a level of $p < 0.05$. The average quantitative BOLD signals were determined in the voxels/clusters showing significant changes per study stimulus by using the thresholded statistical maps as a mask to determine the mean BOLD signal per subject.

### MRI data analysis network functional connectivity changes

Functional network analysis was performed on the same ICA-AROMA preprocessed data using the Beckmann resting state functional networks templates for the default mode and executive control network [38]. The Beckmann auditory network was used as a template for the salience network as this standard template encompasses largely the same brain areas [42]. Brain areas regarded to compose the default mode network according to these templates are; the posterior parietal cortex at the occipito-parietal junction, the pre-cuneus and posterior cingulate cortex, and the frontal pole. Brain areas regarded to compose the executive control network; superior and middle prefrontal cortices, anterior cingulate and paracingulate gyri, and ventrolateral prefrontal cortex, and thalamus. Brain areas regarded to compose the salience network; the lateral superior temporal gyrus and posterior insular cortex, and in the anterior cingulate cortex, anterior supramarginal gyrus and thalamus. Functional connectivity of each network of interest was calculated using the dual regression approach of FSL [46]. This resulted in 3D images for each individual, with voxel-wise Z-scores representing the functional connectivity to each network. The average Z-scores per network were calculated for the pre- and post- ingestion time point. Differences in Z-scores between pre- and post-ingestion were analyzed using paired samples t-tests per functional connectivity network, and uncorrected $p$-value of $p < 0.05$ was deemed significant.

### MRI data analysis eigenvector centrality changes

ECM is an assumption- and parameter-free method to determine the level and quality of connectivity on a voxel-wise level which has been shown to be modulated by the physiological state of the subject and could thus be used to investigate states of hunger satiety and changes in response to nutrient ingestion [44,45]. For the ECM connectivity analysis, the data-driven ICA-based Automatic Removal of Motion Artifacts (ICA-AROMA) was used to identify components in the data related to head

motion and to remove these using linear regression [48,49]. Voxel-based connectivity eigenvector centrality maps were calculated on the ICA-AROMA preprocessed data for each participant using fast-ECM software, which estimates voxel-wise eigenvector centralities from fMRI time series [45]. Pre- and post-ingestion eigenvector centrality maps were compared in a voxel-wise approach in the masked gray matter using the Randomize tool with a paired samples approach and using TFCE [47]. Pre- and post-ingestion scans were compared per condition with paired two-sided contrasts. The same family wise error (FWE) correction at $p < 0.05$ as for the whole brain BOLD analysis was used. The average Quantitative Eigenvector centrality value was determined in the voxels/clusters showing significant changes per study stimulus by using the thresholded statistical maps as a total mask to determine the mean Eigenvector values per subject.

## Results

### Subject characteristics and VAS scores

Subject characteristics for the study group ($n = 20$) are shown in Table 1. No differences were found in subjective scores of satiation at baseline between conditions (Table 2) (VAS hunger $p = 0.805$, VAS fullness $p = 0.141$, VAS wanting meal $p = 0.588$). After ingestion the VAS score for 'wanting to drink more' was similar between all conditions both after first tasting ($p = 0.236$) and after 20 min after full ingestion ($p = 0.259$). All conditions led to a significant decrease in VAS score for 'wanting to continue consumption' from the first tasting to 20 min after full ingestion (glucose $p = 0.002$, fructose $p = 0.004$, sucralose $p = 0.014$, allulose $p = 0.001$).

**Table 1.** Subject characteristics.

| | N = 20 |
|---|---|
| Age (years) | 22.2 ± 1.3 |
| Height (m) | 1.84 ± 0.04 |
| Weight (kg) | 75.6 ± 4.9 |
| BMI (kg/m$^2$) | 22.4 ± 1.1 |

Values in mean ± standard deviation.

**Table 2.** VAS scores for satiation and wanting.

| | Glucose | Fructose | Sucralose | Allulose |
|---|---|---|---|---|
| Hunger pre ingestion | 5.2 ± 2.2 | 5.7 ± 2.3 | 5.7 ± 2.2 | 5.2 ± 2.2 |
| Fullness pre ingestion | 3.0 ± 1.2 | 2.2 ± 1.2 | 3.1 ± 1.5 | 3.0 ± 1.5 |
| Wanting meal pre ingestion | 5.5 ± 2.2 | 5.9 ± 2.7 | 6.5 ± 2.0 | 5.7 ± 2.1 |
| Wanting to continue consumption after tasting | 4.8 ± 1.7 | 5.4 ± 2.2 | 4.5 ± 1.8 | 4.1 ± 2.1 |
| Wanting further consumption after complete ingestion | 3.6 ± 1.9 | 4.2 ± 1.9 | 3.4 ± 2.2 | 2.9 ± 2.0 |

Values in mean ± standard deviation.

### Whole brain BOLD signal changes

Whole brain group average analysis showed significant decreases in BOLD intensity after ingestion of the glucose and the fructose drink (Figure 2, blue color). The sucralose and allulose conditions did not lead to any significant changes in BOLD intensity. Brain areas with decreased activity after glucose ingestion included: the posterior cingulate cortex, brainstem, VTA, insula, lingual and fusiform gyrus. Brain areas that showed decreased activity after fructose ingestion included the anterior cingulate cortex, nucleus accumbens, putamen, and insula. Fructose affected the more frontal parts of the brain, specifically the anterior division of the cingulate cortex instead of the posterior division.

### Network connectivity changes

Analysis of network connectivity (Figure 3) showed that in the salience network, which is associated with feeding behavior and reward, glucose ingestion led to a significant increase in Z-score ($p = 0.048$). Although none of

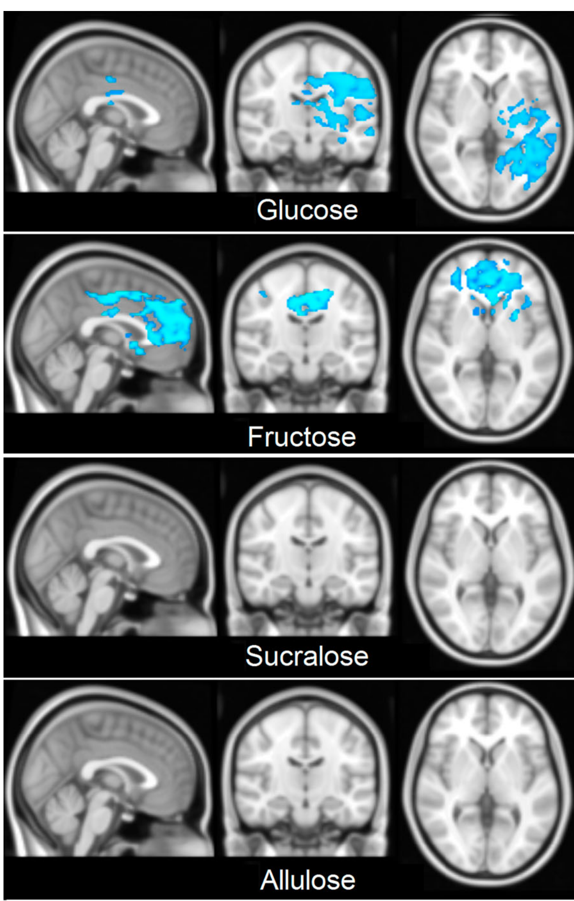


**Figure 2.** Group average BOLD intensity decreases after ingestion of nutrients shakes with the different sweeteners (FWE corrected). Areas with decreases ($P < 0.05$, TFCE, FWE-corrected) in BOLD signal are shown in blue scale.

the effects were significant, the connectivity of the executive control network, involved in decision making, was increased by the glucose sweetened shake while the other shakes led to a decrease in connectivity in this network. The default mode network was not to significantly affected by any of the shakes.

### Eigenvector centrality changes

Figure 4 shows the significant group average brain changes in eigenvector centrality values, indicative of voxel based connectivity, after ingestion of the study stimuli. After ingestion of the glucose sweetened shake, an increase in eigenvector centrality values (red-yellow color scale) was found in various brain areas including the pre- and post-central gyri, posterior cingulate cortex, central opercular cortex and insula, lingual gyrus and other gyri in the occipital lobe. A significant decrease (blue color) in eigenvector centrality values was found in a small area in the anterior brainstem above the pons in proximity to the hypothalamus and VTA. After ingestion of the sucralose stimulus, a significant increase in eigenvector centrality values was found in the medial part of the cingulate cortex and in the pre- and post-central gyrus. A small decrease in eigenvector centrality values was found in the temporal lobe. The allulose and fructose stimuli did not lead to any significant changes in eigen vector centrality values.

### Correlation between quantitative functional MRI measures and subjective scores of hunger and satiety and ratings of test products

The mean BOLD signal in the voxels showing a significant response to glucose was significantly correlated with the VAS score for wanting a meal ($r = 0.477$, $p = 0.034$).

The mean BOLD signal in the voxels showing a significant response to fructose was significantly correlated with the VAS score for wanting a meal ($r = 0.587$, $p = 0.006$). Additionally, the mean BOLD signal was correlated with the VAS score for hunger before ingestion ($r = 0.493$, $p = 0.027$).

The z-score in the salience network after glucose ingestion was correlated with the wanting to continue consumption after tasting ($r = 0.493$, $p = 0.027$). The z-scores in the salience and sensory motor network were significantly correlated with wanting to continue consumption after tasting fructose ($r = 0.527$, $p = 0.017$ and $r = 0.464$, $p = 0.039$ respectively) and the z-score in the salience network was significantly correlated with the VAS score for wanting a meal ($r = -0.498$, $p = 0.025$). The z-score in the default mode network after allulose stimulus ingestion was significantly correlated with the VAS scores for hunger ($r = 0.519$, $p = 0.019$) and wanting a meal ($r = 0.445$, $p = 0.049$) at baseline.

Functional brain measures after sucralose stimulus were not correlated with any of the subjective VAS scores given by the participants.

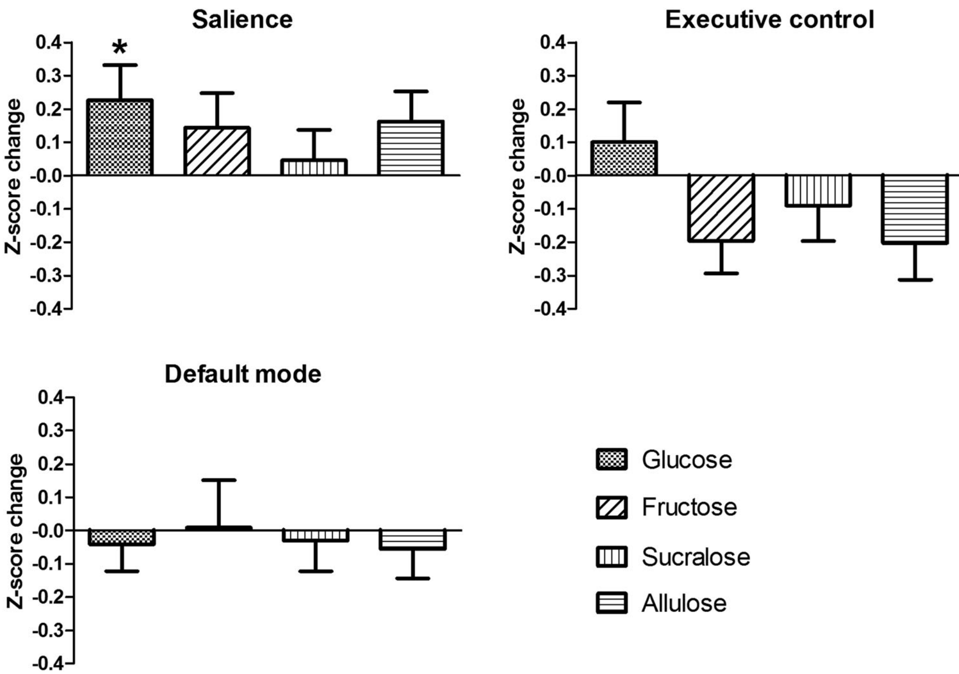


**Figure 3.** Group average changes in network functional connectivity before and after ingestion of the nutrient shakes. Network connectivity changes (mean Z-score per network) per condition are shown for the salience, default mode and executive control functional connectivity network. * indicates a significant difference from baseline $p < 0.05$.

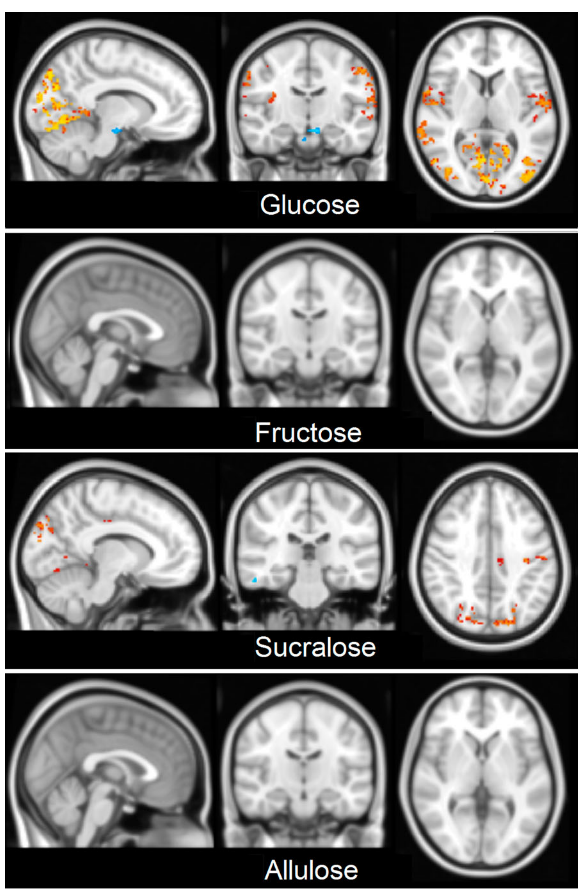


**Figure 4.** Group average eigenvector centrality changes after ingestion of nutrients shakes with the different sweeteners Increases in Eigenvector centrality values are shown in red-yellow scale, decreases are shown in blue scale ($P < 0.05$ TFCE, FWE-corrected).

## Discussion

Our results show that consumption of nutrient shakes sweetened with nutritive sweeteners glucose and fructose had various effects on functional brain response while the shakes sweetened with non/low-nutritive sweeteners sucralose and allulose had little to no effects on functional brain responses. After consumption of the glucose shake, decreased brain activity (decreased BOLD signal), and increased neuronal connectivity both on a voxel based and on a network level was observed. Consumption of the fructose shake led to a decrease in BOLD signal, whereas no changes in neuronal connectivity were observed. Consumption of an allulose or sucralose shake did not lead to any changes in BOLD signal nor in network connectivity. Consumption of the sucralose shake did lead to a small decrease in eigenvector centrality.

Consumption of the glucose sweetened shake results in a decreased BOLD signal in basal brain regions like the posterior cingulate cortex, brainstem, VTA and insula, which is concordant with earlier findings [16]. Our data confirm previous findings that brain activity is diminished after receiving glucose, whereas in a fasted state these brain regions were active in seeking reward or seeking energy [13,14,50]. Additionally, glucose led to a small decrease in voxel based connectivity in the midbrain comprising the hypothalamus and VTA. The hypothalamus and VTA are both strongly involved in homeostatic and hedonic regulation of energy balance [1,3]. Although our data are not fully conclusive, it might be that a decrease in eigen vector centrality in specifically these regions are associated with a decreased state of hunger and increased feeling of satiation [44,51]. More obviously, consumption of the glucose sweetened shake led to a widespread increase of connectivity, both on voxel and network level. Glucose was the only condition which led to a significant increase in connectivity in the salience network. The salience network is involved in feeding behavior and determining reward [41,42], but also in emotional arousal and decision making [52,53]. The increased voxel based and possibly network connectivity in executive control areas, which is involved in decision making [42,43], after glucose ingestion suggests that executive control processes are sensitive to glucose. Our findings are in line with the finding that glucose metabolism is a critical signal regulating response to food cues [54].

The fructose sweetened shake led to a decreased BOLD signal in the anterior cingulate cortex, which is important in executive control and feeding behavior, in addition, several areas involved in the reward response show decreased activity. However, more basal brain areas like the hypothalamus, VTA and other midbrain areas that showed a response to glucose, did not show a decrease in activity after fructose ingestion. This indicates that fructose might not have homeostatic and satiation effects which might affect feeding behavior [25,55]. We expected that intake of fructose would lead to similar effects on the voxel based connectivity as glucose since both are natural nutritive sweeteners and both showed a decrease in the neuronal activity. However, we found no significant effects of the fructose sweetened shake on the eigen vector centrality. This might be explained by the fact that fructose effects on brain connectivity are delayed because fructose is metabolized in the liver and does not deliver energy directly to the brain [56]. Therefore, these effects might not be detectable at the time point (15 min after ingestion) measured in the current study. None of the sugars or sweeteners seemed to have any effect on DMN network connectivity, indicating that the baseline connectivity of the brain is not immediately effected by ingestion of these sweeteners [39,40].

The sucralose and allulose sweetened shakes both showed no significant effect on the BOLD signal. This indicates that these shakes, with very little to no energy content in the form of carbohydrates, had no immediate effect on the activity of brain areas involved in feeding behavior, even though the fat and protein in the shake delivered a significant amount of energy. This indicates that sweet taste without the presence of carbohydrates does not lead to the activity changes as measured with fMRI that are often associated with satiety. While the addition of sweet taste to energy content does strengthen the reward response to energy ingestion [57] earlier findings show that sweet taste without energy content does not lead to a lasting decrease in hypothalamic activity [17], which is in line with our current findings

In contrast to the changes in activity, voxel based connectivity changes were not limited to the carbohydrate containing shakes as the sucralose sweetened shake also demonstrated a significant effect. Increase in voxel based connectivity after sucralose could be seen as processing of the opposing effects in the brain caused by the registration of sweet taste but without corresponding energy content [58–61]. This could also explain the absence of a similar effect in response to the allulose sweetened shake, as allulose is absorbed and does have some energy content. Additionally, the changes in ECM to the sucralose sweetened shake could also indicate a response caused by the fats and protein in the shake as these are the only source of energy in this stimulus and all the other shakes have at least some carbohydrate content. In addition to glucose sensing neurons, the brain has nutrient sensing neurons for fatty acids and amino acids [4,5,62]. Sensing of the different macronutrients separately leads to different brain and downstream peripheral responses [11]. Responses to ingestion of combinations of macronutrients differ from the response to individual substances as the body and the brain responds differently to sugars when ingested in the context of other nutrients [5]. For instance the combination of carbohydrates and fats has been shown to have a stronger effect on the reward system then each of the macronutrients separately [63]. Additionally, sugars are metabolized differently in the context of other nutrients, fructose for example has been shown to be metabolized differently when ingested combined with glucose in a mixed meal than when ingested in isolation [56], which could also alter the response of the brain to its ingestion. The effects of the combination of macronutrients could also explain why we found different changes in connectivity in response to glucose in the context of a mixed meal then when dissolved in water in our earlier study [16].

When looking at the participants subjective rating of satiety through Visual Analogue Scale scoring we found that VAS for wanting a meal and those for hunger correlated with functional brain measures for the glucose, fructose and allulose stimuli but not for the sucralose stimulus. This indicates that the level of satiety can affect the brain response to energy ingestion. This in line with earlier findings showing that states of hunger and satiety have different effects on brain activity [64]. Unfortunately, we did not record feelings of satiety of the participants after ingestion and can therefore not correlate changes in satiety with the changes in brain activity and connectivity.

A limitation of our study is that it was performed only in a very homogenous group of male volunteers. Since it is known that there are several sex-specific differences in energy metabolism [65], it can be expected that sex differences in the brain responses measured in this study are present. Additionally, since no bio chemical data were collected we could not correlate our functional brain responses to changes in blood levels in response to energy ingestion, which could be a driving factor of the response to nutritive sweeteners. Although the crossover design of our study would be suitable for a between treatments comparison, our current study was not powered sufficiently to detect to expectedly more subtle changes between treatment as compared to the pre versus post effects examined here. However, the crossover design was also a strength of our study as it led to homogeneity of the study groups across treatments. A further strength was the hypothesis free approach taking into account the whole brain changes versus a region of interest approach that makes assumptions beforehand.

Our findings show that even in a mixed meal, different types of sweeteners can elicit different brain responses. Therefore the type of sweetener used might differentially affect feeding behavior. Glucose had a widespread effect on the brain. Fructose did affect brain function, but the effect was limited to neuronal activity. Both sucralose and allulose led to very little functional brain responses even though allulose is a mono-saccharide, indicating that sweet taste without the corresponding energy content in these non-nutritive appears to have much smaller effects on the brain then nutritive sweeteners. Taken together, our results show that the ingestion of nutritive sugars elicits a reaction from the brain and could have effects on feeding behavior, both in a satiety aspects as well as rewarding aspects. Because of the little to no effects elicited by the ingestion of non-nutritive sweeteners, these substances might not have effects on feeding behavior, neither positive or negative. Therefore, when regarding the regulation of energy balance and feeding behavior non-nutritive sweeteners could be

used as neutral replacements for nutritive sugars, because while they lack a satiety and rewarding effect they also do not seem to affect other brain responses related to feeding behavior.

## Acknowledgements

AMvO collected the data, did the literature search, wrote the report and made part of the figures. AMvO, AAvdB and AH analyzed the data. AMvO, AAvdB, AH and JvdG interpreted the data. AH prepared part of the figures. JvdG, AAvdB, MH, TPJM, SARRB and AMvO designed the study. All authors critically appraised and edited the report, and approved the manuscript before submission.

## Disclosure statement

MH and TPJM are both employees of Unilever Research and Development Vlaardingen B.V. The Netherlands. All other authors have no conflict of interest to disclose.

## Funding

This study was funded by Unilever Research and Development Vlaardingen B.V. The Netherlands. Unilever markets food and drink products.

## Notes on contributors

*Anna M Van Opstal* is senior researcher at the department of Radiology of the LUMC.

*Anne Hafkemeijer* is senior researcher at the department of Radiology of the LUMC.

*Annette A. van den Berg-Huysmans* is senior researcher at the department of Radiology of the LUMC.

*Marco Hoeksma* is researcher employed by Unilever R&D.

*Theo. P. J Mulder* is researcher employed by Unilever R&D.

*Hanno Pijl* is professor at department of Internal Medicine, Section Endocrinology of the LUMC.

*Serge A. R. B. Rombouts* is professor at the department of radiology of the LUMC and the Institute of Psychology, department of Methodology and Statistics of the Leiden University.

*Jeroen van der Grond* is senior researcher at the department of Radiology of the LUMC.

## ORCID

*Marco Hoeksma* http://orcid.org/0000-0002-3777-5198
*Theo. P. J. Mulder* http://orcid.org/0000-0001-8720-2155

## References

[1] Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature. 2006;443(7109):289–95.
[2] Volkow ND, Wise RA, Baler R. The dopamine motive system: implications for drug and food addiction. Nat Rev Neurosci. 2017;18(12):741–52.
[3] Volkow ND, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. Trends Cogn Sci. 2011;15(1):37–46.
[4] Carreiro AL, Dhillon J, Gordon S, Higgins KA, Jacobs AG, McArthur BM, et al. The macronutrients, Appetite, and energy intake. Annu Rev Nutr. 2016;36:73–103.
[5] Tulloch AJ, Murray S, Vaicekonyte R, Avena NM. Neural responses to macronutrients: hedonic and homeostatic mechanisms. Gastroenterology. 2015;148(6):1205–18.
[6] Imamura F, O'Connor L, Ye Z, Mursu J, Hayashino Y, Bhupathiraju SN, et al. Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: systematic review, meta-analysis, and estimation of population attributable fraction. Br J Sports Med. 2016;50(8):496–504.
[7] Kit BK, Fakhouri TH, Park S, Nielsen SJ, Ogden CL. Trends in sugar-sweetened beverage consumption among youth and adults in the United States: 1999–2010. Am J Clin Nutr. 2013;98(1):180–8.
[8] Malik VS, Schulze MB, Hu FB. Intake of sugar-sweetened beverages and weight gain: a systematic review. Am J Clin Nutr. 2006;84(2):274–88.
[9] Steinert RE, Frey F, Topfer A, Drewe J, Beglinger C. Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal satiety peptides. Br J Nutr. 2011;105(9):1320–8.
[10] Mergenthaler P, Lindauer U, Dienel GA, Meisel A. Sugar for the brain: the role of glucose in physiological and pathological brain function. Trends Neurosci. 2013;36(10):587–97.
[11] Grayson BE, Seeley RJ, Sandoval DA. Wired on sugar: the role of the CNS in the regulation of glucose homeostasis. Nat Rev Neurosci. 2013;14(1):24–37.
[12] Galgani J, Ravussin E. Energy metabolism, fuel selection and body weight regulation. Int J Obesity. 2008;32(Suppl 7):S109–19.
[13] Smeets PA, de Graaf C, Stafleu A, van Osch MJ, van der Grond J. Functional MRI of human hypothalamic responses following glucose ingestion. Neuroimage. 2005;24(2):363–8.
[14] Smeets PA, Vidarsdottir S, de Graaf C, Stafleu A, van Osch MJ, Viergever MA, et al. Oral glucose intake inhibits hypothalamic neuronal activity more effectively than glucose infusion. Am J Physiol Endocrinol Metab. 2007;293(3):E754–E8.
[15] van Opstal AM, vdB-H AA, Hoeksma M, Blonk C, Pijl H, Rombouts SARB, et al. The effect of consumption temperature on the homeostatic and hedonic responses to glucose ingestion in the hypothalamus and the reward system. Am J Clin Nutr. 2018;107(1):20–5.
[16] van Opstal AM, Hafkemeijer A, van den Berg-Huysmans AA, Hoeksma M, Blonk C, Pijl H, et al. Brain activity and connectivity changes in response to glucose ingestion. Nutr Neurosci. 2018 May 27:1–8.
[17] van Opstal AM, Kaal I, van den Berg-Huysmans AA, Hoeksma M, Blonk C, Pijl H, et al. Dietary sugars and non-caloric sweeteners elicit different homeostatic and

hedonic responses in the brain. Nutrition. 2019 April;60:80–86.
[18] Barinaga M. Neuroscience: what makes brain neurons run? Science. 1997;276(5310):196–8.
[19] Grill HJ, Berridge KC, Ganster DJ. Oral glucose is the prime elicitor of preabsorptive insulin secretion. Am J Physiol. 1984;246(1 Pt 2):R88–R95.
[20] Lam CK, Chari M, Lam TK. CNS regulation of glucose homeostasis. Physiology (Bethesda). 2009;24:159–70.
[21] Ulrich M, Endres F, Kolle M, Adolph O, Widenhorn-Muller K, Gron G. Glucose modulates food-related salience coding of midbrain neurons in humans. Hum Brain Mapp. 2017;16(1):55.
[22] Lohner S, Toews I, Meerpohl JJ. Health outcomes of non-nutritive sweeteners: analysis of the research landscape. Nutr J. 2017;16:55.
[23] Tappy L, Le KA. Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev. 2010;90 (1):23–46.
[24] Page KA, Chan O, Arora J, Belfort-Deaguiar R, Dzuira J, Roehmholdt B, et al. Effects of fructose vs glucose on regional cerebral blood flow in brain regions involved with appetite and reward pathways. JAMA. 2013;309 (1):63–70.
[25] Purnell JQ, Fair DA. Fructose ingestion and cerebral, metabolic, and satiety responses. JAMA. 2013;309(1):85–6.
[26] Rogers PJ, Hogenkamp PS, de Graaf C, Higgs S, Lluch A, Ness AR, et al. Does low-energy sweetener consumption affect energy intake and body weight? A systematic review, including meta-analyses, of the evidence from human and animal studies. Int J Obes (Lond). 2016;40 (3):381–94.
[27] Swithers SE. Artificial sweeteners produce the counterintuitive effect of inducing metabolic derangements. Trends Endocrinol Metab. 2013;24(9):431–41.
[28] Fantino M, Fantino A, Matray M, Mistretta F. Beverages containing low energy sweeteners do not differ from water in their effects on appetite, energy intake and food choices in healthy, non-obese French adults. Appetite. 2018;125:557–65.
[29] Oshima H, Kimura I, Izumori K. Psicose contents in various food products and its origin. Food Sci Technol Res. 2006;12(2):137–43.
[30] Sclafani A, Zukerman S, Ackroff K. Postoral glucose sensing, not caloric content, determines sugar reward in C57BL/6J mice. Chem Senses. 2015;40(4):245–58.
[31] Noronha JC, Braunstein CR, Glenn AJ, Khan TA, Viguiliouk E, Noseworthy R, et al. The effect of small doses of fructose and allulose on postprandial glucose metabolism in type 2 diabetes: A double-blind, randomized, controlled, acute feeding, equivalence trial. Diabetes Obes Metab. 2018;20(10):2361–70.
[32] Chung MY, Oh DK, Lee KW. Hypoglycemic health benefits of D-psicose. J Agric Food Chem. 2012;60 (4):863–9.
[33] Morton GJ, Meek TH, Schwartz MW. Neurobiology of food intake in health and disease. Nat Rev Neurosci. 2014;15(6):367–78.
[34] Forster BB, MacKay AL, Whittall KP, Kiehl KA, Smith AM, Hare RD, et al. Functional magnetic resonance imaging: the basics of blood-oxygen-level dependent (BOLD) imaging. Can Assoc Radiol J. 1998;49(5):320–9.
[35] Smeets PA, de Graaf C, Stafleu A, van Osch MJ, van der Grond J. Functional magnetic resonance imaging of human hypothalamic responses to sweet taste and calories. Am J Clin Nutr. 2005;82(5):1011–6.
[36] Vidarsdottir S, Smeets PA, Eichelsheim DL, van Osch MJ, Viergever MA, Romijn JA, et al. Glucose ingestion fails to inhibit hypothalamic neuronal activity in patients with type 2 diabetes. Diabetes. 2007;56(10):2547–50.
[37] Rombouts SA, Scheltens P, Kuijer JP, Barkhof F. Whole brain analysis of T2* weighted baseline FMRI signal in dementia. Hum Brain Mapp. 2007;28(12):1313–7.
[38] Beckmann CF, DeLuca M, Devlin JT, Smith SM. Investigations into resting-state connectivity using independent component analysis. Philos Trans R Soc Lond B Biol Sci. 2005;360(1457):1001–13.
[39] Tregellas JR, Wylie KP, Rojas DC, Tanabe J, Martin J, Kronberg E, et al. Altered default network activity in obesity. Obesity (Silver Spring, Md). 2011;19 (12):2316–21.
[40] Kullmann S, Heni M, Veit R, Ketterer C, Schick F, Haring HU, et al. The obese brain: association of body mass index and insulin sensitivity with resting state network functional connectivity. Hum Brain Mapp. 2012;33 (5):1052–61.
[41] Garcia-Garcia I, Jurado MA, Garolera M, Segura B, Sala-Llonch R, Marques-Iturria I, et al. Alterations of the salience network in obesity: a resting-state fMRI study. Hum Brain Mapp. 2013;34(11):2786–97.
[42] Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, et al. Dissociable intrinsic connectivity networks for salience processing and executive control. J Neurosci. 2007;27(9):2349–56.
[43] Bolo NR, Musen G, Simonson DC, Nickerson LD, Flores VL, Siracusa T, et al. Functional connectivity of insula, basal ganglia, and prefrontal executive control networks during Hypoglycemia in type 1 diabetes. J Neurosci. 2015;35(31):11012–23.
[44] Lohmann G, Margulies DS, Horstmann A, Pleger B, Lepsien J, Goldhahn D, et al. Eigenvector centrality mapping for analyzing connectivity patterns in fMRI data of the human brain. PLoS One. 2010;5(4):e10232.
[45] Wink AM, de Munck JC, van der Werf YD, van den Heuvel OA, Barkhof F. Fast eigenvector centrality mapping of voxel-wise connectivity in functional magnetic resonance imaging: implementation, validation, and interpretation. Brain Connect. 2012;2(5):265–74.
[46] Jenkinson M, Beckmann CF, Behrens TE, Woolrich MW, Smith SM. FSL. Neuroimage. 2012;62(2):782–90.
[47] Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage. 2009;44(1):83–98.
[48] Pruim RH, Mennes M, van Rooij D, Llera A, Buitelaar JK, Beckmann CF. ICA-AROMA: a robust ICA-based strategy for removing motion artifacts from fMRI data. Neuroimage. 2015;112:267–77.
[49] Pruim RH, Mennes M, Buitelaar JK, Beckmann CF. Evaluation of ICA-AROMA and alternative strategies for motion artifact removal in resting state fMRI. Neuroimage. 2015;112:278–87.
[50] Trivedi BP. Neuroscience: dissecting appetite. Nature. 2014;508(7496):S64–S5.

[51] Wright H, Li X, Fallon NB, Crookall R, Giesbrecht T, Thomas A, et al. Differential effects of hunger and satiety on insular cortex and hypothalamic functional connectivity. Eur J Neurosci. 2016;43(9):1181–9.

[52] Uddin LQ. Salience processing and insular cortical function and dysfunction. Nat Rev Neurosci. 2015;16 (1):55–61.

[53] Menon V, Uddin LQ. Saliency, switching, attention and control: a network model of insula function. Brain Struct Funct. 2010;214(5):655–67.

[54] de Araujo IE, Lin T, Veldhuizen MG, Small DM. Metabolic regulation of brain response to food cues. Current Biology: CB. 2013;23(10):878–83.

[55] Purnell JQ, Klopfenstein BA, Stevens AA, Havel PJ, Adams SH, Dunn TN, et al. Brain functional magnetic resonance imaging response to glucose and fructose infusions in humans. Diabetes Obes Metab. 2011;13(3):229–34.

[56] Theytaz F, de Giorgi S, Hodson L, Stefanoni N, Rey V, Schneiter P, et al. Metabolic fate of fructose ingested with and without glucose in a mixed meal. Nutrients. 2014;6(7):2632–49.

[57] Veldhuizen MG, Babbs RK, Patel B, Fobbs W, Kroemer NB, Garcia E, et al. Integration of sweet taste and metabolism Determines carbohydrate reward. Curr Biol. 2017;27(16):2476–85. e6.

[58] Davidson TL, Martin AA, Clark K, Swithers SE. Intake of high-intensity sweeteners alters the ability of sweet taste to signal caloric consequences: implications for the learned control of energy and body weight regulation. Q J Exp Psychol (Hove. 2011;64 (7):1430–41.

[59] Davidson TL, Swithers SE. A Pavlovian approach to the problem of obesity. Int J Obes Relat Metab Disord. 2004;28(7):933–5.

[60] Swithers SE, Davidson TL. A role for sweet taste: calorie predictive relations in energy regulation by rats. Behav Neurosci. 2008;122(1):161–73.

[61] Fowler SP. Low-calorie sweetener use and energy balance: results from experimental studies in animals, and large-scale prospective studies in humans. Physiol Behav. 2016;164(Pt B):517–23.

[62] Magnan C, Levin BE, Luquet S. Brain lipid sensing and the neural control of energy balance. Mol Cell Endocrinol. 2015;418(Pt 1):3–8.

[63] DiFeliceantonio AG, Coppin G, Rigoux L, Edwin Thanarajah S, Dagher A, Tittgemeyer M, et al. Supra-additive effects of combining fat and carbohydrate on food reward. Cell Metab. 2018;28(1):33–44. e3.

[64] Führer D, Zysset S, Stumvoll M. Brain activity in hunger and satiety: an exploratory visually stimulated fMRI study. Obesity. 2008;16(5):945–50.

[65] Varlamov O, Bethea CL, Roberts CT Jr. Sex-specific differences in lipid and glucose metabolism. Front Endocrinol (Lausanne). 2014;5:241.