Functional brain responses in the maintenance of energy balance

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CHAPTER 5

The effect of consumption temperature on the homeostatic and hedonic responses to glucose ingestion in the hypothalamus and the reward system

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ABSTRACT

Background

Excessive consumption of sugar sweetened beverages (SSBs) has been associated with obesity and related diseases. SSBs are often consumed cold, both the energy content and temperature might influence the consumption behavior of SSBs.

Objective

The main aim of this study is to elucidate whether consumption temperature and energy (i.e., glucose) content modulate homeostatic (hypothalamus) and reward (VTA) responses.

Design: Sixteen healthy men participated in our study (aged 18-25, BMI 20-23). High resolution fMRI data was collected after ingestion of four different study stimuli; plain tap water at room temperature (22°C), plain tap water at 0°C, a glucose containing beverage (75g of glucose dissolved in 300 ml water) at 22°C, and a similar glucose drink at 0°C. BOLD changes from baseline (7 mins pre-ingestion) were analyzed over time in the hypothalamus and VTA for individual stimulus effects and for effects between stimuli.

Results

In the hypothalamus, water at 22°C led to a significantly increased BOLD response, all other stimuli resulted in a direct, significant decrease in BOLD response compared to baseline. In the VTA, a significantly decreased BOLD response compared to baseline was found after ingestion of stimuli containing glucose at 0°C and 22°C. These responses were not significantly modulated by consumption temperature. Drinking plain water did not have a significant VTA BOLD effect.

Conclusions

Our data show that glucose at 22°C, glucose at 0°C, and water at 0°C lowered hypothalamic activity, which is associated with increased satiation. On the contrary, drinking of water at room temperature increased activity. All stimuli led to a similar VTA response suggesting that all drinks elicited a similar hedonic response. Our results indicate that in addition to glucose, the low temperature at which sweetened beverages are often consumed also leads to a response from the hypothalamus and might strengthen the response of the VTA.

Keywords: Hypothalamus, glucose, energy sensing, ventral tegmental area, temperature, reward system.

INTRODUCTION

The global prevalence of obesity has risen over the past three decades [1]. An important contributor to this increase, among other factors, is an escalation of consumption of added sugars, especially in the form of sugar sweetened beverages (SSBs)[2-4]. Several factors are involved in the palatability and rewarding aspect of consumption of SSBs, including the energy content because of the sugars and refreshment and thirst quenching because of the low consumption temperature. Consumption of sugars has been shown to have an effect on reward areas in the brain in rodents [5-7], which could lead to continued overconsumption of sugar, although these effects are still unclear in humans [8]. In addition to the high sugar content, the relatively low temperature at which SSBs are generally consumed could intensify the reward responses as cold drinks are more thirst quenching than drinks consumed at room temperature.[9]

In the brain, the hypothalamus regulates both energy expenditure and intake and thirst [10,11]. Glucose containing beverages have been shown to alter the functional hypothalamic BOLD (Blood Oxygen Level Dependent) response proportionally with the energy (glucose) content of the beverage consumed [12]. It is currently not known how the temperature of a consumed beverages or sugar sweetened beverage relates to regulation of energy and fluid homeostasis. In addition to these homeostatic interactions, hedonic aspects of SSBs are important determinants of consumer behavior. Brain areas involved in this hedonic response are the ventral tegmental area (VTA) and areas of the limbic system (amygdala, nucleus accumbens). Although BOLD responses in these regions are consistently linked to reward [13], little is known about the effects of the BOLD response in these reward centers caused by energy content and drinking beverages at different temperatures. In addition to the unknown interaction of energy and temperature in the hypothalamus, the relationship between the temperature at which beverages are consumed and hedonic brain responses is unknown. As mentioned previously, such interactions can be expected since SSBs are less appreciated at room temperature, than cooled [14].

Taken together, there are reasons to believe that consumption temperature has an effect on brain responses regarding energy regulation and hedonic reward, and hence may impact energy intake in humans. Furthermore, it seems likely that energy homeostasis and hedonic aspects are interdependent. In the present study we aim to elucidate the effects of two aspects of SSB ingestion, namely the energy content and ingestion temperature, on homeostatic and reward response. To this end, we studied the effects of plain water and glucose containing water at both room temperature and at 0^{o}C, on brain reward (VTA) and homeostatic (hypothalamus) centers, in a double blind, four-way crossover study.

METHODS

Subjects

Sixteen healthy, normal-weight, Caucasian young adult men participated in our study. Subjects were recruited via local advertisements and through the use of mailing lists. A participant flow chart for the recruitment of subjects is include as Supplemental Figure 1. All subjects were between the ages of 18 and 25 and had a body mass index (BMI) ranging from 20 to 23. Exclusion criteria were the following: a history of diabetes or disturbed glucose metabolism; any genetic, psychiatric, renal, hepatic or other chronic or current disease; recent fluctuations in weight > 3 kg; current smoking; current alcohol consumption >21 units/week and use of recreational drugs; recent blood donation or participation in other biomedical research (within the last 3 months); use of medication affecting glucose or lipid metabolism; contra-indications for MRI-scanning. Written informed consent was obtained from all subjects and the protocol was approved by the Medical Ethics Committee of the Leiden University Medical Centre. The study protocol is registered at ClinicalTrails.gov under registration number NCT03181217.

Experimental procedure

We used a randomized, controlled, crossover study design. Randomization was based on a Williams Design for four study stimuli in four periods, balanced for periods and carry over, randomization was performed by an independent person at Unilever R&D by generating a randomized list of stimulus order per subject. The four different study stimuli used were; water at room temperature (22°C) and 0°C, and glucose containing beverages at room temperature (22°C) and at 0°C. All beverages consisted 300 ml of water with or without 75 g of glucose added. Before all study visits participants were fasted from 10.00 pm the previous day but were allowed to drink water. During each study visit after 7 minutes of baseline fMRI scanning the study stimuli were administered during the fMRI scan through a peroral tube, lying supine in the scanner, in the 32-channel head coil. Subjects were instructed to drink the total amount in a steady and continuous way. Scanning was continued during and after start of ingestion. The hypothalamus was used as a seed region to determine the homeostatic response to ingestion of the study stimuli and as a post-hoc analysis the VTA was used as a seed region to determine the hedonic response.

Blood sampling

Blood samples were used to ascertain a normal glucose metabolism in each subject. On every test day, 2 blood samples (5 ml each) were drawn by venipuncture in an

antecubital vein; one sample was taken before scanning and the other one after the scanning procedure, 30 minutes after ingestion of the study stimuli. Sample handling and analysis was performed by the laboratory for Clinical Chemistry at Leiden University Medical Center. Plasma glucose was measured using a fully automated Hitachi 704/911 system. Plasma insulin was measured by a Radio Immuno Assay (Medgenix, Fleurus, Belgium). A total amount of 10 ml blood was taken on each study day.

MRI data acquisition and analysis

MRI was performed on a 3.0 Tesla Achieva clinical scanner (Philips Healthcare, Best, the Netherlands). A mid sagittal (x=0) T1 structural hypothalamus scan (single slice scan, repetition time 550 ms, echo time 10 ms, field of view 208x208 mm, voxelsize = 0.52x0.52x14 mm, scan time 1.14 min) and a mid-brain single slice (x=0) T_2* weighted, gradient echo-planar imaging (EPI) fMRI scan that renders BOLD contrast (repetition time 120 ms, echo time 30 ms, field of view 208x208 mm, voxelsize = 0.81 x 0.81 x 14 mm, scan time 21.2 min, 500 dynamics) were made. Imaging data were pre-processed and analyzed using Functional Magnetic Resonance Imaging of the Brain Software Library (FSL) version 5.0.8. Data was pre-processed as described in earlier studies [15]. Data were averaged for each set of 4 subsequent volumes, reducing the 500 dynamic scans to 125. The hypothalamus was segmented manually on the middle volume of the single slice MRI scan according to anatomical landmarks as previously described [15] (average number of voxels: 155), the VTA ROI was also segmented manually in similar manner using the mammillary bodies and the cerebral aqueduct as anatomical anchor points (average number of voxels: 51) (example ROIs shown in figure 1). To correct for scanner drift, all hypothalamic BOLD values were corrected for the BOLD signal obtained in an internal reference ROI. This internal reference ROI was drawn in grey matter, superior of the genu of the corpus callosum. To establish the post-ingestion hypothalamic BOLD response to the stimuli, the mean baseline signal (first 7 minutes of scan time) was measured for contrast. All data points (n=125) were divided by the mean baseline value and converted to percentages, yielding the percentage signal change relative to baseline.

Statistical analysis

Statistical analysis was performed using SPSS version 23.0.0.2. To analyze differences in blood values between the different study stimuli we used repeated measures ANOVA. All fMRI results are reported as percentage BOLD change relative to baseline (0-7 minute pre-drinking BOLD response). For presentation purposes, data were pooled into 'minute' data points: The first seven minutes were considered baseline BOLD response. Data between minute 8 and 11 were omitted from the analysis due to non-

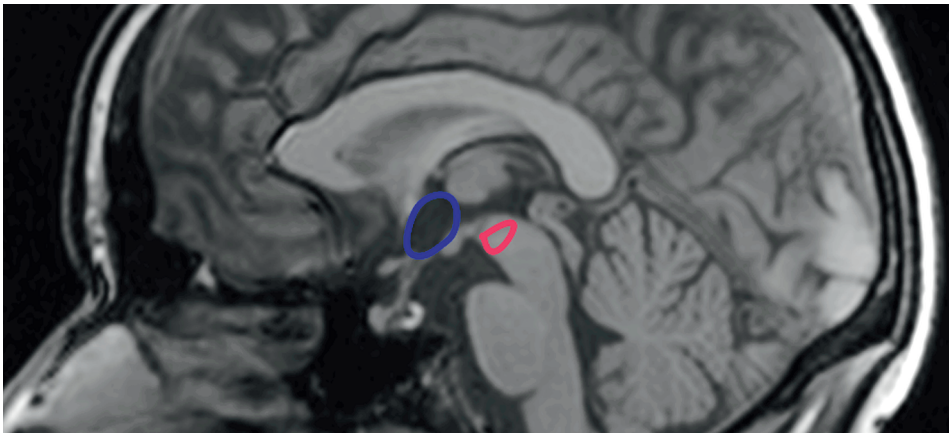

Figure 1. Region of interest (ROIs) used for the BOLD response analysis. Hypothalamus ROI indicated in blue and ventral tegmental area (VTA) ROI indicated in red.

physiologic excessive BOLD changes caused by swallowing of the test drink (BOLD change of more than 3.5% at 3T)[16]. Data from minute 11 and up (11-21) were considered the post drinking BOLD response. To determine whether study stimuli led to a significant BOLD change, repeated measures ANOVA was used per study stimulus, in which the data for each stimulus was pooled per minute (by averaging bins of 6 consecutive fMRI volumes for each minute). Statistical analysis for the comparison of the stimuli effect between the study stimuli was performed by a mixed model analysis using the study stimulus as a fixed effect, time point (125 time points per subject per treatment) as a covariate and subject per occasion as a random factor. Additionally, to analyze potential differences in timing of the response of the individual stimuli, the post drinking BOLD response was analyzed per minute using the same mixed model but with the per minute time points as a factor instead of a covariate. Uncorrected p-values, due to small sample size, of <0.05 are deemed significant for all tests.

RESULTS

Subject characteristics

Baseline subject characteristics are shown in Table 1. Blood levels for glucose and insulin showed a normal glucose metabolism for all participants with a normal response to the glucose stimuli. Furthermore, all participants had normal fasting insulin and glucose blood levels, confirming that all participants were fasted on the study days.

Table 1. Subject characteristics

	n=16
Age (years)	20.2 ± 1.7
Height (m)	1.81 ± 0.05
Weight (kg)	71.8 ± 4.9
BMI (kg/m^2)	22.0 ±1.15
Glucose Fasted[1]	5.0 ± 0.3
Post glucose ingestion[1]	7.0 ± 1.0
Insulin Fasted[1]	4.8 ± 2.4
Post glucose ingestion[1]	25.2 ± 17.0

Values in mean ± standard deviation. Glucose levels in mmol/L, Insulin levels in mU/L.
[1] average blood levels for the two visits with glucose stimuli per subject (n=16), post ingestion levels measured 30 minutes after ingestion.

No significant differences (p>0.05) in absolute and relative blood values were found between the different glucose stimuli.

Changes in hypothalamic activity

Figure 2 shows the BOLD response of the hypothalamus over time per study stimulus. General inspection of the response curve shows that the hypothalamic BOLD response after ingestion of water at 22ºC remained positive during the entire experiment, whereas the BOLD response after ingestion of water at 0º, or glucose at 22ºC or 0ºC normalized after a few minutes. Table 2 shows the average hypothalamic post ingestion BOLD response relative to baseline for all study stimuli. Water at 22°C led to a significant average increase (1.2%, p=0.027) in BOLD response. Water at 0°C, glucose at 22°C and 0°C all resulted in a decrease in BOLD response relative to baseline. Figure 2 shows that directly after ingestion, a significant decrease in BOLD that returned to baseline was observed for all three of these stimuli, as indicated by the asterisks (maximum difference water 22°C: 1.5% p=0.009, and at 0°C: -2.0% p=0.014, glucose at 22°C: -1.9% p=0.011 and at 0°C: -2.2% p=0.005), although the average decrease over the entire post ingestion period was not significant. When comparing the BOLD responses (after intervention) to the water response at 22ºC, water at 0°C, glucose at 22°C and 0°C all showed an significant average BOLD decrease (mean difference of -1.6%, -1.6% and -1.8% respectively, all p<0.05) compared to water at 22ºC (table 3).

Changes in Ventral Tegmental Area activity

Figure 3 shows the BOLD responses of the VTA over time for all stimuli. Both glucose stimuli (glucose at 22°C and 0°C) showed a significantly decreased BOLD signal relative

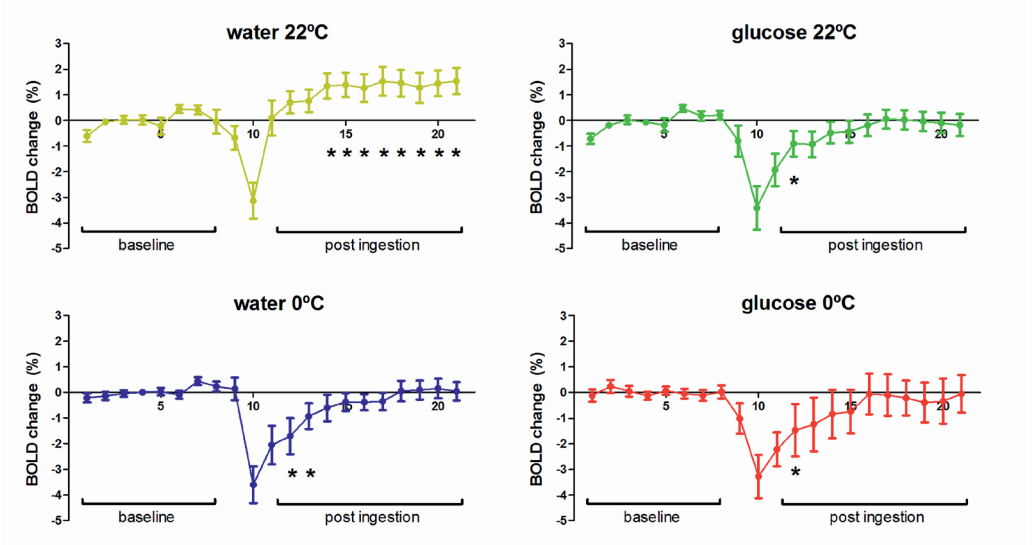

Figure 2. Hypothalamic BOLD response. BOLD responses to all four study stimuli as group average (n=16) response per minute with standard errors. Study stimuli were ingested from minute 8-10, overall response period post ingestion of study stimulus from minute 11-21. * Post ingestion BOLD response significantly different from baseline period at p<0.05 (repeated measures ANOVA).

Table 2. Average post-ingestion BOLD response relative to baseline (n=16)

	Hypothalamic response (%)		**VTA response (%)**	
	Mean ± SE	p-value[1]	*Mean ± SE*	p-value[1]
Water 22°C	+1.2 ± 0.5	0.027	-0.6 ± 0.4	0.150
Water 0°C	-0.5 ± 0.3	0.133	-0.7 ± 0.5	0.203
Glucose 22°C	-0.4 ± 0.4	0.300	-0.6 ± 0.6	0.348
Glucose 0°C	-0.7 ± 0.8	0.387	-1.5 ± 1.0	0.154

[1] Determined by repeated measures ANOVA analysis

Table 3. Average post-ingestion BOLD response relative to Water 22°C control condition (n=16)

	Hypothalamic response (%)		VTA response (%)	
	Mean difference ± SE	p-value[1]	*Mean difference ± SE*	p-value[1]
Water 0°C	-1.6 ± 0.7	0.028	-0.2 ± 0.9	0.831
Glucose 22°C	-1.6 ± 0.8	0.040	-0.1 ± 1.0	0.898
Glucose 0°C	-1.8 ± 0.7	0.019	-0.8 ± 0.9	0.388

[1] Determined by linear mixed model analysis

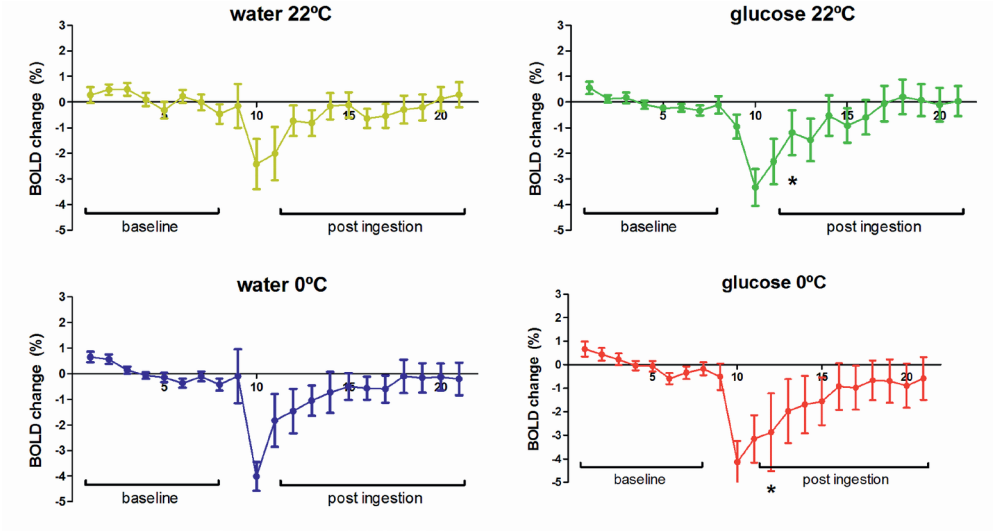

Figure 3. Ventral tegmental area BOLD response. BOLD responses to all four study stimuli as group average (n=16) response per minute with standard errors. Study stimuli were ingested from minute 8-10, overall response period post ingestion of study stimulus from minute 11-21. * Post ingestion BOLD response significantly different from baseline period at p<0.05 (repeated measures ANOVA).

to baseline directly after ingestion (maximum difference: glucose at 22°C -0.5% p=0.0 and at 0°C -0.5% p=0.0) but did not result in an average significant VTA BOLD change compared to baseline over the entire post-ingestion time (p>0.1, table 2). Both water stimuli did not have a significant effect on the VTA BOLD signal, over the entire post-ingestion time (p>0.1, table 2) or directly after ingestion (maximum difference: water at 22°C: -2.2% p=0.055 and at 0°C: -1.6% p=0.093). Although the VTA BOLD response after ingestion of glucose at 0°C appears most pronounced, no statistically significant differences were found between stimuli when all four responses were compared with mixed model analysis (p>0.3 for all stimuli, table 3). In all cases, the VTA BOLD response normalized after a few minutes.

DISCUSSION

Our data show that drinking water at 0°C, even in the absence of glucose, resulted in a significant decrease in hypothalamic BOLD response. Drinking of glucose at both ambient and cold temperature conditions also resulted in a significant decrease in the hypothalamic BOLD signal. This finding is in line with previous research indicating that the hypothalamus is a key brain structure for energy sensing [12]. On the contrary, when compared to the pre-drinking baseline, drinking water at 22°C resulted in a significant increase of hypothalamic BOLD response that lasted for at least 12 minutes. In the VTA, all stimuli led to an immediate decrease in BOLD signal. Although both glucose stimuli (glucose at 22°C and 0°C) showed a significantly decreased BOLD response in the VTA immediately after ingestion, this response was not significantly modulated by consumption temperature. All VTA BOLD responses normalized to baseline within the time frame of the study.

The hypothalamus plays a central role in the regulation of temperature, fluid homeostasis and thirst and control of energy intake and feeding behavior [11,17,18]. To regulate energy intake and feeding behavior the hypothalamus relies on various complex neural systems that together regulate satiety signaling and hunger cues [19]. In a fasted and thus "hungry" state, these neural systems are highly active to drive feeding behavior [20,21]. When activity in these systems is suppressed, the drive for feeding is decreased, indicating that a decrease in activity in the hypothalamus could be a marker of satiation. Similar response are found during thirst and thirst quenching [10,22]. Our data show that hypothalamic activity decreases in response to both the low temperature stimulus and glucose stimulus in a similar manner. Water at room temperature was seen to increase hypothalamic activity. Our results indicate that the low temperature at which SSB are most often consumed in and of itself can lead to a satiety, or more likely thirst quenching, response from the hypothalamus. Although, water at low temperature elicits a response from the hypothalamus, no temperature effects were found on consumption of glucose containing stimuli, therefore our data do not suggest that temperature has an additive effect.

The VTA is known to be a core structure involved in hedonic motivation for energy intake [23]. Earlier studies have shown that in a fasted state, food cues lead to VTA activation [24,25]. In addition, intravenous infusion of glucose modulates the reward response in the VTA [26]. Our data show that the VTA also responds directly to the ingestion of glucose. In addition to the response to energy intake, the activation of the VTA by oral temperature might be important since it is known that the hedonic dimension of thermal sensation in humans also motivates energy intake in order to maintain core temperature [27]. The response to ingesting glucose at 0°C seemed to

strengthen the average response in the VTA by 0.7% compared to drinking glucose at 22°C. Although the response was not significantly different between both stimuli at might suggest an interplay between temperature and energy content in the response of the reward system.

A limitation of our study is that it was performed in male volunteers only and it can be expected that sex differences are present, since it is known that there are several sex-specific differences in energy metabolism [28]. A second limitation of our study design is the lack of subjective ratings of hunger, thirst and satiety and regular consuming habits. Combining our more objective fMRI measurements with these subjective measures would give more insight into the satiety- and hedonic response. Thirdly, our participants were fasted, which may limit the extrapolation of results to a non-fasted state. Also, we used a high dose glucose stimulus, to be able to generalize our results to everyday consumption future studies have to be done using other sweeteners more commonly used in SSBs in a lower dose. Additionally, as we only found few significant results due a relatively large variation in VTA responses between participants an increase in sample size would be preferable to be able to better determine the VTA response and the difference in response between stimuli. Furthermore, it should be noted that various publications have reported large changes in BOLD in the reward system resulting from visual food cues and listening to music enjoyable music, indicating that ingestion of energy is not necessary for a neurophysiological reward responses [24,29]. A strength of our study was the use of the specific high resolution mid brain fMRI scan, which allowed us to accurately determine ROIs and perform BOLD measurements in the hypothalamus and VTA [12,13].

In conclusion, our results indicate that low temperature of consumption can elicit a homeostatic response. In the hypothalamus, we observed a decrease in BOLD signal immediately after intake of cold and glucose containing drinks, whereas the signal was increased after intake of room temperature water. Although suppression of the hypothalamic BOLD signal is often associated with satiation, and increased BOLD signal with hunger and drive for feeding, a lack of subjective hedonic and satiety measures prevents us from analyzing this further. Future research should focus on investigating the effects of cold SSB on the VTA and hypothalamus in an extended, more heterogeneous study population with males and females and including groups at risk for becoming obese from regular consumption of SSBs, such as children and adolescents [30].

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Author contributions JvdG, AvdBH, MH and CB designed research; AvO and JvdG conducted research; AvO and AvdBH analyzed data; AvO and JvdG wrote the paper; JvdG had primary responsibility for final content. All authors read and approved the final manuscript

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