Glucose Modulates Food-Related Salience Coding of Midbrain Neurons in Humans

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Abstract: Although early rat studies demonstrated that administration of glucose diminishes dopaminergic midbrain activity, evidence in humans has been lacking so far. In the present functional magnetic resonance imaging study, glucose was intravenously infused in healthy human male participants while seeing images depicting low-caloric food (LC), high-caloric food (HC), and non-food (NF) during a food/NF discrimination task. Analysis of brain activation focused on the ventral tegmental area (VTA) as the origin of the mesolimbic system involved in salience coding. Under unmodulated fasting baseline conditions, VTA activation was greater during HC compared with LC food cues. Subsequent to infusion of glucose, this difference in VTA activation as a function of caloric load leveled off and even reversed. In a control group not receiving glucose, VTA activation during HC relative to LC cues remained stable throughout the course of the experiment. Similar treatment-specific patterns of brain activation were observed for the hypothalamus. The present findings show for the first time in humans that glucose infusion modulates salience coding mediated by the VTA. *Hum Brain Mapp* 37:4376–4384, 2016. © 2016 Wiley Periodicals, Inc.

Key words: ventral tegmental area; glucose modulation; functional magnetic resonance imaging; salience coding; reward; mesolimbic system; obesity

INTRODUCTION

In the past decade, the increasing prevalence of obesity [Ng et al., 2014] has motivated different research lines elucidating the neural mechanisms regulating feeding behavior. Particularly, functional neuroimaging in humans has identified brain regions that may be considered as either

homeostatic or nonhomeostatic (hedonic) systems [e.g., Gibson et al., 2010]. Homeostatic feeding is signaled by caloric needs and is mediated by the hypothalamus sensing the internal energy status [Berthoud and Morrison, 2008]. Hedonic eating can be driven by high-caloric (HC) food cues, triggering an anticipatory signal of pleasure and the consumption of food, even in the absence of caloric need. In the long run, energy intake will exceed energy expenditure, eventually leading to the accumulation of body fat [Hill et al., 2012].

Modulation of homeostatic feeding is particularly mediated by the arcuate nucleus of the hypothalamus and the lateral hypothalamic area, whereas nonhomeostatic feeding behavior is driven by mesolimbic brain regions, with the midbrain's ventral tegmental area (VTA) as its core structure. While the mesolimbic system has traditionally been related to reward processing, its functions may now be best summarized as coding motivational value and

Additional Supporting Information may be found in the online version of this article.

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motivational salience, given that mesolimbic brain regions are also involved in processing aversive, nonrewarding information [Bromberg-Martin et al., 2010]. One of the central mechanisms of coding salience is the increase in firing rates of dopaminergic midbrain neurons, mediated by switching from tonic to phasic burst activity [Wenzel and Cheer, 2014].

Neuroimaging studies in humans have shown that food cues activate the VTA [Beaver et al., 2006; Kroemer et al., 2013; Malik et al., 2008; Murdaugh et al., 2012; Ochner et al., 2011; Schur et al., 2009; Siep et al., 2012; Stoeckel et al., 2008]. Of note, activity of dopaminergic midbrain neurons is glucose-responsive. In studies with rats, local or systemic administration of glucose to the substantia nigra or the VTA diminished the firing rate of dopaminergic neurons [Amoroso et al., 1990; During et al., 1995; Freeman et al., 2001; Levin, 2000; Saller and Chiodo, 1980]. As a putative mechanism, down-regulation of dopaminergic midbrain activity due to increased glucose levels is possibly mediated by inhibitory input from γ -amino-butyric acid neurons, whose excitability increases following K-ATP channel activation [Amoroso et al., 1990; During et al., 1995]. Complementary, it is also conceivable that dopaminergic midbrain neurons might be hyperpolarized more directly by glucose-induced increase of Na^+/K^+ ATPase activity, similar to hypothalamic glucose-inhibited neurons (see Discussion).

At the systems level, several mostly resting state neuroimaging studies without any functional challenge have investigated the effects of glucose administered by ingestion, by intragastric infusion, or by intravenous infusion [Little et al., 2014; Liu et al., 2000; Luo et al., 2015; Page et al., 2013; Smeets et al., 2005a, 2005b, 2007, 2011; Vidarsdottir et al., 2007], often focusing on the hypothalamus as the sole region of interest (ROI) [Liu et al., 2000; Smeets et al., 2005a,b, 2007]. These studies have revealed that the hypothalamus responds with decreased overall activity upon administration of glucose.

However, in contrast to the homeostatic system, it is largely unexplored whether glucose also modulates dopaminergic midbrain activation in humans. A recent study conducted by Little et al. (2014) reported glucose-induced down-modulation of midbrain activity dorsally, possibly in the vicinity of the locus coeruleus or the parabrachial nuclei, but not in the VTA. Thus, despite early evidence from animal work that dopaminergic midbrain neurons are susceptible to glucose modulation [Amoroso et al., 1990; During et al., 1995; Freeman et al., 2001; Levin, 2000; Saller and Chiodo, 1980], to our knowledge, this has not yet been confirmed using *in vivo* neuroimaging in humans.

We therefore used functional magnetic resonance imaging (fMRI) to investigate the effects of glucose on VTA activation in healthy participants. To experimentally elicit a VTA response, we used food cues because food is a primary reinforcer, and human fMRI studies have repeatedly shown that food cues reliably activate the VTA [Beaver

et al., 2006; Kroemer et al., 2013; Malik et al., 2008; Murdaugh et al., 2012; Ochner et al., 2011; Schur et al., 2009; Siep et al., 2012; Stoeckel et al., 2008]. In the present study, a food/non-food (NF) discrimination task was performed by two groups of participants. One group received 10 g intravenous glucose infusion after a fasting baseline period of saline infusion while discriminating between food and NF stimuli. Food cues were either low-caloric food (LC) or HC. We hypothesized that VTA activation would be higher to HC relative to LC food cues during the initial baseline period. After infusion of glucose, this HC minus LC difference in VTA activity was expected to decrease. To control that the treatment (e.g., the dose of infused glucose) was sufficient, activation of the VTA was additionally compared with that of the hypothalamus which has consistently been shown to decrease its activity in response to ingested or infused glucose [Smeets et al., 2005a,b, 2007]. A second group of participants completed the same food/NF task in the absence of glucose administration to provide information about the stability of the HC minus LC difference over time under unmodulated, fasting baseline conditions.

MATERIALS AND METHODS

Participants

Fourty-two healthy male medical students were recruited from the local university, and randomly assigned to two experimental groups. The group receiving glucose (glucose group) consisted of 24 participants with a mean age of 24.6 years (standard deviation [SD] = 3.0 years) and a mean body mass index (BMI) of 23.6 kg/m^2 (SD = 2.1 kg/m^2). The control group (no glucose infusion) comprised 18 volunteers aged 23.5 years on average (SD = 2.8 years) with a mean BMI of 24.0 kg/m^2 (SD = 3.2 kg/m^2). Groups did not differ in age and BMI (age: $t(40) = 1.18$, $P = 0.245$; BMI: $t(40) = 0.42$, $P = 0.675$). According to self-reports, participants' body weight was stable for at least three months before the start of the study. There were no reports of psychiatric/neurological disorders, disturbed glucose tolerance resulting from insulin resistance or diabetes, or any other contraindications regarding the infusion of glucose or the fMRI procedure. Written informed consent was obtained prior to the experiment. The study was approved by the local ethics committee at the University of Ulm, and was in accordance with the Declaration of Helsinki.

fMRI Task

All participants performed a discrimination task on visual LC food (e.g., apple, celery, salad) and HC food (e.g., pizza, spare ribs, Viennese Schnitzel) vs. neutral NF pictures (e.g., candle, flower, dog), taken from the food-pics database (<http://www.food-pics.sbg.ac.at/>) [Blechert et al., 2014]. Example stimuli are shown in Figure 1A. A total of 240

Figure 1.

(A) Six example stimuli, taken from the food-pics database [Blechert et al., 2014]. (B) Schematic overview of the fMRI experiment which consisted of four inherent experimental phases with 6.4 min duration each, not noticeable to participants. After phase 1, participants of the glucose group received 10 g glucose dissolved in 50 ml saline, intravenously infused within 30 s. The control group did not receive any infusion. 15 LC and 15 HC food cues as well as 30 NF pictures were presented per phase. The task during fMRI was to discriminate between food and NF pictures.

stimuli were used for the main experiment: 60 LC stimuli, 60 HC stimuli, and 120 NF stimuli. An additional practice run comprised further 20 stimuli (5 × LC, 5 × HC, and 10 × NF). The NF stimuli were ensured not to be related to food, that is, pictures of kitchen utensils or food packaging were not included. Participants were instructed that “whole animals” were considered as NF, whereas prepared parts of animals, for example, spare ribs, were regarded as food stimuli. Mean energy content per 100 g depicted food was 130 kJ (SD = 76 kJ, range: 38 kJ–352 kJ) for LC food stimuli, and 1,357 kJ (SD = 620 kJ, range: 656 kJ–2,738 kJ) for HC stimuli. The average total number of kJ displayed per image was 316 kJ (SD = 293 kJ) for LC, and 3,047 kJ (SD = 2,635 kJ) for HC stimuli. Apart from differing in energy content per 100 g ($t(118) = 15.22, P < 0.001$) and in total number of kJ displayed per image ($t(118) = 7.98, P < 0.001$), other parameters such as physical features (e.g., saturation) and subjective ratings (e.g., arousal) were matched as far as possible. Averaged parameters associated with LC, HC, and NF pictures as well as their respective database indices are given in Supporting Information Tables 1 and 2, respectively.

Because we were interested in obtaining information on the time course of glucose-induced modulation of brain activation in the glucose group, the experiment was divided into four phases of equal length (6.4 min/phase; Fig. 1B) with 15 LC, 15 HC, and 30 NF trials per phase. Participants were not aware of this subdivision. For all phases, trials were ordered according to optimized trial sequences obtained using the program “Optseq2” (<http://surfer.nmr.mgh.harvard.edu/optseq/>) [see also Dale, 1999]. In a post-processing step, the entire trial sequence was modified to allow not more than three repetitions of a specific trial

type, and onsets were jittered by randomly adding fractions of the fMRI repetition time. During the experiment, in accordance with the prespecified trial order, stimuli were randomly assigned to the four phases. Each specific stimulus was only presented once per participant. Food and NF stimuli appeared in the center of a white background for 2.5 s each. Participants were instructed to press two predefined keys with their right index or middle finger when the picture represented food or NF, respectively. Responses had to be given as fast and as accurately as possible. During the intertrial periods (average length = 3.8 s, SD = 3.9 s, maximum = 25.0 s), a centered black cross was shown which participants were asked to fixate. Stimuli were presented on a 32" LCD display (NordicNeuroLab AS, Bergen, Norway), projected through the scanner bore, and then reflected by a mirror, mounted on the MRI coil. Software used for stimulus delivery was Presentation 14.8 (Neurobehavioral Systems, San Francisco), running under Windows 7 Professional SP1 on a 64 bit standard PC.

Procedure

All participants were asked to refrain from eating or drinking HC beverages after 7:00 p.m. the day before the experiment. Participants arrived at the laboratory the next morning at 7:30 or 9:00 a.m., when their fasting state was tested by measuring the blood glucose level using a mobile glucose meter (ACCU-CHEK Inform II; Roche Diagnostics GmbH, Mannheim, Germany). This also ensured that participants of the glucose group would tolerate subsequent infusion of 10 g glucose, an amount well below the recommended maximum dose of 35 g [Bingley et al., 1992]. After careful instruction, written informed consent was obtained. In the glucose group only, blood was collected from the antecubital vein of the left arm for the analysis of several blood parameters (for details, please refer to Supporting Information Table 3). Under fMRI conditions, all participants performed a practice run of the food/NF discrimination task before the main experiment was performed which lasted 25.6 min. About 6.4 min after the start of the experiment, participants of the glucose group received 50 ml of 20% glucose solution (B. Braun Melsungen AG, Melsungen, Germany), infused into the already prepared venous access using an MRI-compatible infusion pump (ACCUTRON MR; MEDTRON AG, Saarbrücken, Germany). Infusion of glucose lasted 30 s, corresponding to a flow rate of about 1.7 ml/s. Before and after the administration of glucose, saline solution (0.9% NaCl; B. Braun Melsungen AG, Melsungen, Germany) was infused at a flow rate of 0.1 ml/s. Participants of the glucose group were told they would receive either a solution of glucose or saline, depending on a “randomization scheme,” but in fact, all participants of the glucose group were treated identically as described above. During debriefing after MR scanning, participants were informed about the deception.

Volunteers of the control group did not receive glucose or saline infusions, nor was blood collected, although the fasting blood glucose level was determined prior to the experiment using the mobile glucose meter. This was mainly because experiments involving the control group were performed two months earlier than those of the glucose group, with the infusion pump not yet in place at that time, but scanning time already scheduled.

MRI Data Acquisition

MRI was performed on a 3 Tesla MAGNETOM Prisma in combination with a 64 channel head/neck coil (Siemens AG, Erlangen, Germany). First, a high resolution T1-weighted structural image was acquired using a magnetization prepared rapid acquisition gradient echo sequence with following parameters: Repetition time (TR) = 2,300 ms, echo time (TE) = 2.96 ms, inversion time = 900 ms, flip angle = 9°, bandwidth = 240 Hz/Px, PAT factor = 2 (GRAPPA mode), field of view (FOV) = 256 mm, matrix size = 256 × 256, voxel volume = 1 mm³, slice orientation: sagittal; scan time about 5.5 min).

Functional images measuring the T2*-weighted blood oxygen level-dependent signal were obtained using an echo-planar pulse sequence (EPI) with TR = 2,000 ms, TE = 33 ms, flip angle = 90°, bandwidth = 2,136 Hz/Px, PAT factor = 2 (GRAPPA mode), FOV = 220 mm, matrix size = 90 × 90, ascending slice acquisition yielding 32 transversal slices, slice thickness = 3.0 mm, interslice gap = 1.0 mm, voxel size = 2.44 mm × 2.44 mm × 4.00 mm. Scan time was about 25.6 min, corresponding to 768 EPI volumes.

Subsequent to the experiment, a T2 proton density (PD)-weighted scan was performed with the following parameters: TR = 5,550 ms, TE = 21 ms, flip angle = 120°, bandwidth = 326 Hz/Px, PAT factor = 2 (GRAPPA mode), FOV = 288 mm, matrix size = 384 × 384, ascending slice acquisition, 41 transversal slices, slice thickness = 1.8 mm, interslice gap = 0.22 mm, voxel size = 0.75 mm × 0.75 mm × 2.02 mm, scan time about 4.5 min).

MRI Data Preprocessing and Analysis

Imaging data preprocessing and statistical analyses were performed with the software package Statistical Parametric Mapping version 12 (r6225, Wellcome Department of Cognitive Neurology, London, UK). Functional EPI images were slice time corrected (reference slice: 16) and spatially realigned to the mean EPI which also served as reference image to which the T1 and PD images were coregistered. The T1 image was then segmented and the resulting forward deformations were used to spatially normalize all images to the Montreal Neurological Institute (MNI) space. EPI images were smoothed using a Gaussian kernel with 8 mm full width at half maximum. After preprocessing, all images had a voxel size of 2 mm × 2 mm × 2 mm.

Preprocessed functional data were modeled using a standard hierarchical approach. First, for every participant a general linear model was set up. Twelve separate regressors were formed for phase-specific, correctly responded LC, HC, and NF trials by specifying their respective onsets. In addition, incorrect trials and the spatial realignment parameters were added to the design matrix as regressors of no interest. Resulting delta functions were convolved with the canonical hemodynamic response function and its time and dispersion derivatives. To remove low-frequency scanner drifts, data were high-pass filtered (cutoff: 128 s), and an autoregression model of polynomial order 1 was used to account for temporally correlated residual errors.

On single-subject model estimation, one-sided *t*-contrast images were computed that represented the averaged, estimated magnitude of neural activation associated with LC, HC, and NF (against implicit baseline) of the four phases. The contrast images were submitted to a random-effects analysis (flexible factorial design) with factors Subject, Treatment (glucose vs. no treatment), and a third factor representing the concatenated factors Phase and Condition with 12 levels (Ph1_LC, Ph1_HC, Ph1_NF, Ph2_LC, Ph2_HC, Ph2_NF, Ph3_LC, Ph3_HC, Ph3_NF, Ph4_LC, Ph4_HC, and Ph4_NF).

From a recent study on glucose-induced modulation of brain activity [Jin et al., 2014], we estimated that the maximal effect of glucose administration on VTA activity would be achieved about 6 min to 10 min post glucose infusion when applying a dose of 10 g glucose, corresponding to about 0.10 g to 0.15 g glucose per kg body weight. Thus, we predicted VTA activity to be maximally modulated by glucose in experimental phase 3 which covered minutes 6.4 to 12.8 subsequent to glucose infusion. Therefore, we tested for a significant Treatment by Phase by Condition interaction using a directional *t*-contrast: greater HC minus LC difference in unmodulated phase 1 relative to modulated phase 3 [Ph1_HC - Ph1_LC - (Ph3_HC - Ph3_LC)] between both groups (glucose minus control). The contrast was applied to the VTA and to the hypothalamus, both defined as ROIs on an anatomical basis. The VTA ROI (volume: 134 voxels or 1,072 mm³) was manually delineated on transversal slices (MNI z-coordinates: -18 to -6; Supporting Information Fig. 1), relying on the group-averaged PD image loaded in MRIcron (<http://www.mricron.com/mricron/>) [see also Rorden et al., 2007]. The hypothalamus was defined as a second ROI using the WFU PickAtlas 2.4 [Lancaster et al., 1997, 2000; Maldjian et al., 2003, 2004]. The hypothalamic ROI consisted of 21 voxels (168 mm³). Because analysis was confined to the ROIs, statistical parametric maps were assessed at an uncorrected voxel height threshold of $P < 0.05$.

Analysis of Reaction Times

Motivated by the neuroimaging results, suggesting a reversal in the desire to eat energy-dense food by infusion

Figure 2.

Treatment by Phase by Condition interaction reflecting a greater difference in neural activation elicited by high-caloric minus low-caloric food cues for phase 1 relative to phase 3 for the glucose-treated group vs. the untreated control group. Depicted are significant voxels within the ROIs covering the VTA (for ROI delineation, see Supporting Information Fig. 1) and the

hypothalamus. Respective mean parameter estimates are shown in Figures 3 and 4. Statistical parametric maps were overlaid on transverse (left) and sagittal (right) sections of a PD image obtained by averaging over all participants ($n = 42$). Coordinates refer to MNI space. L: left; R: right.

of glucose, we investigated whether that effect was also evident at the behavioral level. We inspected reaction times (RTs) for LC and HC cues obtained from the food/NF decisions. Usually, the more rewarding a stimulus, the faster the associated response [Lernbass et al., 2013; Maliejko et al., 2014]. We therefore expected RTs to be faster for HC than for LC food cues under unmodulated conditions (i.e., phase 1), and to change with glucose administration. Accordingly, individual RT differences reflecting the Phase by Condition interaction “Ph1_LC - Ph1_HC - (Ph3_LC - Ph3_HC)” were subjected to a two-sample t -test (glucose group vs. control group).

RESULTS

Blood Glucose Levels

Pre-experimental blood glucose levels as measured by the mobile glucose meter confirmed each participant's fasting state (range: 67 mg/dl – 110 mg/dl). At baseline, the glucose group (mean = 87.1 mg/dl, SD = 6.7 mg/dl) and the control group (mean = 89.8 mg/dl, SD = 9.6 mg/dl) did not significantly differ in blood glucose concentration ($t(40) = 1.07$, $P = 0.289$).

Neuroimaging Results

Analysis of neural activation revealed that in the VTA and in the hypothalamus neural activation was significantly modulated by infusion of glucose. The Treatment (glucose vs. no treatment) by Phase (phase 1 vs. phase 3) by Condition (HC vs. LC) interaction was significant ($P < 0.05$) in 13 voxels of the VTA and in 10 voxels of the hypothalamus (Fig. 2).

Under fasting baseline conditions (phase 1), the glucose group showed numerically higher VTA activation during HC compared with LC food cues (Fig. 3 and Supporting Information Fig. 2). In phase 2 which began with the

administration of glucose and then lasted about 6 min, the magnitude of activation in the VTA for HC food cues numerically decreased, while it remained virtually the same for LC stimuli. In phase 3, VTA activation further numerically decreased for HC cues, and increased for LC stimuli. In phase 4, VTA activation associated with HC cues partially recovered. The control group, not treated with glucose, showed consistently greater VTA activation for HC than for LC stimuli in all phases, and the greatest numerical HC minus LC difference was observed for phase 3.

As can be derived from Figure 4 and Supporting Information Figure 3, the pattern of neural activation in the hypothalamus was in part very similar to that observed for the VTA: The glucose group showed a positive numerical HC minus LC difference only in phase 1. This difference reversed already in phase 2 and was maximally negative in phase 3. Of note, the relative activation level was mostly below zero. In the control group, neural activation evoked by HC food stimuli was numerically higher compared with LC cues in all phases, and the level of overall activation was positive for HC, and negative for LC food cues.

Behavioral Results

There was a significant treatment difference of the Phase by Condition interaction in RTs [Ph1_LC - Ph1_HC - (Ph3_LC - Ph3_HC)]: $t(40) = 1.75$, $P = 0.044$ (one-sided). As expected, mean RTs were numerically faster for the more rewarding HC stimuli than for LC stimuli in both groups during phase 1. In the glucose group, this RT advantage decreased in phase 3, but further increased in the control group (for details, please refer to Supporting Information Fig. 4).

DISCUSSION

In the present human fMRI study, a bolus of intravenously infused glucose was shown to significantly

Figure 3.

Averaged magnitude of neural activation [arbitrary unit] derived from 13 significant voxels of the VTA for the glucose group (left) and the control group (right). Activation was greater for HC (in red) than for LC (in green) food cues during phase 1. After phase 1 when the glucose group received a bolus of 10 g glucose intravenously, the VTA's hemodynamic response

reversed, with greater activation during LC relative to HC food cues. In the untreated control group, VTA activation was consistently higher in response to HC than LC stimuli. Please see also Supporting Information Figure 2 which includes the NF condition. Error bars represent standard error of the mean.

modulate neural responsivity of the VTA in healthy male volunteers. Under fasting baseline conditions, HC food cues elicited higher VTA activation than LC food cues, which is in line with previous research using food cues [Beaver et al., 2006; Kroemer et al., 2013; Malik et al., 2008; Murdaugh et al., 2012; Ochner et al., 2011; Schur et al., 2009; Siep et al., 2012; Stoeckel et al., 2008]. After receiving 10 g glucose, the difference between HC and LC gradually reversed such that the VTA now responded numerically stronger to LC than to HC food stimuli. In a control group not treated with glucose, VTA activation was consistently higher for HC than for LC cues during all experimental

phases. Similar treatment-specific findings were revealed for the hypothalamus. Neural responses in either brain region were mirrored behaviorally by changes in average RTs to LC and HC food cues. To the best of our knowledge, the present study provides the first evidence that glucose alters neural activation of the ventral dopaminergic midbrain in humans. This outcome was expected on the basis of early rat studies demonstrating that local or systemic administration of glucose diminishes the firing rate of dopaminergic neurons in the substantia nigra and the VTA [Amoroso et al., 1990; Daring et al., 1995; Freeman et al., 2001; Levin, 2000; Saller and Chiodo, 1980].

Figure 4.

Magnitude of hypothalamic activation [arbitrary unit] during LC (in green) and HC (in red) food cues averaged across 10 significant voxels. Please see also Supporting Information Figure 3 which includes activation for the NF condition. Error bars represent standard error of the mean.

Most neuroimaging studies, however, have focused on effects of glucose on hypothalamic activation, thus, confirmation of the above cited animal findings in humans had been lacking so far. The present study bridges this gap by showing that also in humans salience coding mediated by the VTA is susceptible to glucose modulation.

Of note, glucose treatment not only leveled off the difference in VTA activation for LC and HC stimuli but reversed this difference so that LC food cues were associated with a significantly ($P < 0.05$) higher level of VTA activity during experimental phase 3. This finding is rather surprising as we expected glucose to suppress VTA activity for both kinds of LC and HC stimuli, with perhaps the more salient HC stimuli to be affected disproportionately. This prediction was derived from the literature, suggesting the existence of specialized glucose-sensing neurons. For instance, hypothalamic subpopulations of glucose-excited neurons rather directly increase their firing rate when the level of glucose rises [Anand et al., 1964; Oomura et al., 1964, 1969]. Conversely, other subpopulations of hypothalamic neurons are excited by lowering glucose concentrations, and are inhibited by elevated glucose levels [Anand et al., 1964; Kurita et al., 2015; Oomura et al., 1964, 1974; Silver and Erecinska, 1998]. Here, the underlying mechanism might involve the hyperpolarizing effect of Na^+/K^+ ATPase, whose activity increases in response to elevated ATP levels, resulting from metabolized glucose through glycolysis and oxidative phosphorylation [Jordan et al., 2010; Kurita et al., 2015; Silver and Erecinska, 1998].

The observed reversal in VTA activation with stronger responses to LC relative to HC food cues during phase 3 suggests that participants' desire to eat may have shifted from HC food to LC food, which also receives support from the RT analyses. While the well-known [e.g., Lernbass et al., 2013; Malejko et al., 2014] response time acceleration for more rewarding or salient HC stimuli remained stable in the control group (no glucose), and was also present in the experimental group before glucose administration, this difference gradually decreased after infusion of the glucose bolus. The entire response pattern certainly deserves further investigation, given that it points to an interesting field of application, the utilization of glucose for treating obesity. The idea appears quite paradoxical at first glance but is not unreasonable when accounting for the molecular mechanisms outlined above. In the modern civilized world, humans have access to an abundance of food, and are heavily faced with cues of tasty food. The mesolimbic system, including the VTA, is designed to be driven by those highly salient stimuli, which may culminate in "hedonic eating," a condition when an individual eats in the absence of caloric needs [Meye and Adan, 2014], which may lead to the development of obesity. In adipose patients, it might therefore be useful to suppress motivational salience of HC food (cues) to avoid or reduce overeating. The present finding that administered glucose

decreases the VTA's response to HC food cues, and even increases the response of the VTA during LC relative to HC food cues, suggests that glucose could perhaps be therapeutically used to facilitate a kind of relearning during therapeutic exposition and extinction, perhaps promoting the consumption of LC food while, at the same time, restricting intake of potentially unhealthy HC food [Demos et al., 2012; García-García et al., 2014; Grosshans et al., 2012; Murdaugh et al., 2012; Stice et al., 2011]. Thus, small doses of glucose — as one part of a comprehensive therapeutic concept, and administered under appropriate conditions — may be suitable to successfully treat obesity much like other drug dependencies [e.g., Xue et al., 2012]. Clearly, this approach needs to be further investigated in future studies with corresponding experimental settings.

A more transient limitation of the present study is that only men were investigated so far, and present results do not yet inform about what to expect from a female group. This research line is currently underway in our laboratory, however, under strict control of humoral alterations during the menstrual cycle, due to its likely influence on the desire to eat energy-dense food [e.g., Alonso-Alonso et al., 2011; Frank et al., 2010; Hormes and Timko, 2011].

Another limiting aspect of the present study is the use of food cues to elicit VTA activation. Both food (cues) and glucose are closely tied, and it would be interesting to investigate whether glucose-mediated modulation of VTA activation also works with other salient stimuli, for example, images of erotic or sexual content, with sex as another primary reinforcer. Also, results from those highly salient but NF stimuli would perhaps help to delineate hypotheses about the underlying mechanism. So far we cannot provide sufficient explanation for the present observations, at least in terms of glucose-sensing neurons which have been reported as the cellular mechanism of glucose-sensing, particularly in the hypothalamus. Indeed, the hypothalamus and the VTA are in close anatomical proximity such that the present activity pattern in both structures might be the result of mutual interaction. Still, if glucose-sensing was the only mechanism of action, one would have expected more uniform activation changes for LC and HC food cues, which was, however, not the case. Rather, the present pattern of activity reversal for LC and HC stimuli before and after glucose appears suggestive that there might exist some kind of local mutual or lateral inhibition of glucose-sensing neurons, as has already been proposed for sensory systems [e.g., Von Békésy, 1967].

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