

## Sucrose activates human taste pathways differently from artificial sweetener

Guido K.W. Frank,<sup>a</sup> Tyson A. Oberndorfer,<sup>b</sup> Alan N. Simmons,<sup>b</sup> Martin P. Paulus,<sup>b</sup> Julie L. Fudge,<sup>c</sup> Tony T. Yang,<sup>b</sup> and Walter H. Kaye<sup>b,d,\*</sup>

<sup>a</sup>University of Colorado at Denver and Health Sciences Center, Department of Psychiatry, The Children's Hospital, 13123 E. 16th Avenue, Aurora, CO 80045, USA

<sup>b</sup>University of California at San Diego, Department of Psychiatry, MC: 0603La Jolla, CA 92093-0603, USA

<sup>c</sup>University of Rochester Medical Center, Department of Psychiatry, 300 Crittenden Boulevard, Rochester, New York, NY 14642-8409, USA

<sup>d</sup>University of Pittsburgh, Department of Psychiatry, Western Psychiatric Institute and Clinic, 3811 O'Hara St., Pittsburgh, PA 15213, USA

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**Animal models suggest that sucrose activates taste afferents differently than non-caloric sweeteners. Little information exists how artificial sweeteners engage central taste pathways in the human brain. We assessed sucrose and sucralose taste pleasantness across a concentration gradient in 12 healthy control women and applied 10% sucrose and matched sucralose during functional magnet resonance imaging. The results indicate that (1) both sucrose and sucralose activate functionally connected primary taste pathways; (2) taste pleasantness predicts left insula response; (3) sucrose elicits a stronger brain response in the anterior insula, frontal operculum, striatum and anterior cingulate, compared to sucralose; (4) only sucrose, but not sucralose, stimulation engages dopaminergic midbrain areas in relation to the behavioral pleasantness response. Thus, brain response distinguishes the caloric from the non-caloric sweetener, although the conscious mind could not. This could have important implications on how effective artificial sweeteners are in their ability to substitute sugar intake.**

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### Introduction

Artificial sweeteners are frequently substituted for sugar with the goal of reducing caloric intake. It is unclear, though, if artificial sweeteners do in fact promote weight loss (Vermunt et al., 2003). While artificial sweetener use was correlated with increased body weight in a large epidemiologic study (Stellman and Garfinkel, 1986; Stellman and Garfinkel, 1988), such a relationship was later disputed (Rolls, 1991). Surprisingly little is known about how artificial sweet taste is processed in the human brain and if there

is a biological difference of central feeding and reward system activation compared to sugar.

Sweet taste perception of both sugars and artificial sweeteners is peripherally mediated by the tongue T1R3 receptor in conjunction with the T1R2 receptor type (Chandrashekar et al., 2006). This sensory information is transmitted via the cranial nerves VII, IX and X to the nucleus tractus solitarius (NTS), from there to the thalamic ventroposterior medial nucleus (VPM) and then to the primary gustatory cortex, which in humans comprises the frontal operculum and the anterior insula (FO/AI) (Ogawa, 1994). The anterior insula is separated from the posterior insula by the central sulcus; the anterior section is further divided into the anterior, middle and posterior short gyri, and recent brain imaging studies have indicated some functional differences for those subunits (Nitschke et al., 2006). The so-called middle insula falls primarily within the posterior short gyrus and has been found to engage when subjects try to detect a particular taste (Veldhuizen et al., 2007).

Artificial sweeteners bind to subunits of the taste receptors with greater affinity (lower dissociation constant) compared to sucrose (Nie et al., 2005). Furthermore, behavioral preference together with gustatory neural response in the absence of T1R3 receptors has been shown for sugars including sucrose but not for artificial sweeteners such as sucralose in a study by Damak et al. (2003). In that study, wild-type and T1R3 knockout (KO) mice were tested for bottle preference of sweetener solution over water, and cranial nerve response was recorded. KO mice showed reduced taste preference compared to the wild-type for sucrose but no preference for sucralose at all. That finding suggested that the T1R3 receptor may be the only sweet taste receptor for artificial sweeteners but that there may be additional receptors that respond to sugars (Damak et al., 2003).

Studying a biologic brain response to those peripheral sensory stimulations, that is not cognitively prejudiced, is complicated. Many artificial sweeteners have an aftertaste that is easily

\* Corresponding author. University of Pittsburgh, Department of Psychiatry, Western Psychiatric Institute and Clinic, 3811 O'Hara St., Pittsburgh, PA 15213, USA. Fax: +1 412 647 9740.

E-mail address: kayewh@upmc.edu (W.H. Kaye).

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detectable, which may induce a cognitive or affective bias toward the substance ingested. The relatively new artificial sweetener sucralose, sold under the trade name “Splenda,” is an ideal example to study since it is widely used, and there is little bitter after taste reported (Schiffman et al., 1995). Sucralose (1,6-dichloro-1,6-dideoxy- $\beta$ -D-fructofuranosyl-4-chloro-4-deoxy- $\beta$ -D-galactopyranoside) is a disaccharide that is made from sucrose that selectively substitutes three atoms of chlorine for three hydroxyl groups in the sugar molecule; it is about 600 times as sweetening compared to sucrose (FDA, 1998). The commercially available product “Splenda” has a small amount maltodextrin added as a volume enhancer. The total calorie amount is considered zero, although a negligible amount of calories are derived from the maltodextrin (FDA, 1998).

Sweet taste perception can be measured for taste quality (such as sweet or bitter), hedonic “liking,” as well as the incentive motivational component “wanting” (Berridge, 1996). Taste pleasantness or “liking” varies with the type of sweet stimulus and varies from person to person (Moskowitz, 1971), but it has been shown to be more state independent compared to “wanting” (Finlayson et al., 2007). It is incompletely understood what structures of the brain reward system circuitry in humans specifically mediate taste pleasantness or “liking.” However, the ventral striatum, an area that is closely connected to the midbrain, has been implicated (Pecina and Berridge, 2005; Tindell et al., 2006). In addition, altered taste preference has been reported after insult to the insula (Kim and Choi, 2002), although bilateral anterior insula lesions did not eliminate the ability to express taste preference altogether (Adolphs et al., 2005).

In this study, we wanted to determine whether human brain activation is different for caloric sucrose compared to an artificial sweetener. We hypothesized that sucrose would elicit greater brain activation compared to the artificial sweetener sucralose (Splenda) in the FO/AI and reward pathways, including the midbrain ventral tegmental area (VTA) and nucleus accumbens (Morton et al., 2006). We felt this was reasonable since, while taste signal from both stimuli get transmitted via the shared TIR3 receptor, receptors specific for the caloric sucrose (Damak et al., 2003) should add to the sucrose-related brain signal. In addition, we wanted to test whether we can measure brain taste–pathway activation for the two sweet taste stimuli using brain imaging that is consistent with the neuroanatomical literature. We hypothesized that both sweeteners would activate primary brain taste centers (FO/AI) and related areas such as cingulate, striatum, thalamus and orbitofrontal cortex. Finally, we wanted to study if individual “liking” or pleasantness for sweet taste predicts brain activation and if there is a subset of the taste pathway that is specific for the pleasantness response. We hypothesized that the insula and the dopaminergic reward-related ventral striatum or the ventral tegmental midbrain might be implicated in this response. If indeed sucralose (Splenda) were to stimulate taste and reward pathways less, this could suggest it might not be as rewarding, resulting in an activated but maybe unsatisfied reward system.

## Materials and methods

### *Study participants and assessments*

Twelve healthy normal weight control women (CW) were recruited through local advertisements and signed a written informed consent to participate in this study. A 13th woman (data

not shown) that was recruited had to be excluded since she could not fulfill the criteria for behavioral matching of sweet solutions (see below, at any concentration tested could she reliably distinguish the two sweet taste types).

Subjects were between 20 and 36 years old (mean  $27 \pm 6$  years), had a current body mass index (BMI, weight in kg/height in  $m^2$ ) between 20 and 25 (mean  $22 \pm 2$ ), with a mean low lifetime BMI of  $21 \pm 1$ , and had a high lifetime BMI of  $23 \pm 2$ . All subjects had normal menstrual cycles and were studied only during the first 10 days of the menstrual cycle since brain reward mechanisms vary depending on the reproductive phase (Dreher et al., 2007).

Clinical interviews and self-assessments were applied in order to assure control status. Subjects were screened and assessed for Axis I psychiatric illness with the Structured Clinical Interview for DSM-IV Axis I disorders (APA, 2000). Trained doctoral level clinicians administered the clinical interviews. The screening procedure indicated that no participant had a history of an eating disorder (ED) or any psychiatric, serious medical or neurological illness. CW were not on medication, including herbal supplements.

In order to avoid sub-threshold disordered eating, we applied the Eating Disorders Inventory-2 (EDI-2; Garner, 1991) that showed normal values for body dissatisfaction (EDI)  $3 \pm 5$ , bulimic symptoms (EDI)  $0.5 \pm 1$  and drive for thinness (EDI)  $1 \pm 2$ .

This study was conducted according to the institutional review board regulations of the University of California San Diego.

### *Behavioral testing*

Two taste tests were administered. In order to assess the sensory and hedonic sweet taste perception, subjects rated on a 9-point scale pleasantness and sweetness of sucrose (Mallinckrodt Chemicals, Phillipsburg, NJ, laboratory grade) solutions, which were made in the concentrations from 0%, 2%, 4%, 8%, 16% and 32% in distilled water. A similar set of sucralose (Splenda<sup>®</sup>, McNeil Nutritionals, Ft. Washington, PA) solutions matched for sweetness was made. Those sucralose (Splenda) solutions were matched to sucrose based on manufacturer conversions: 1 packet of sucralose (Splenda), approximately 1.0 g, is equal to 2 teaspoons of sugar; 10 g of sugar is equal to 2.4 teaspoons of sugar. Accordingly, 3.84 g of sucralose (Splenda) was dissolved in 100 g of distilled water to match the 32% sucrose solution, and 1.92, 0.96, 0.48 and 0.24 g were used to create solutions that matched the sucrose solutions.

To reduce cognitive bias, sucrose and sucralose (Splenda) solutions were given blindly and in random order. The hedonic pleasantness scale ranged from 1, “like not at all,” to 9, “like extremely,” where 5 is “neither like nor dislike.” Sweetness ratings consistently show a positive linear slope with increasing concentration across simple and complex carbohydrates (Moskowitz, 1971). Pleasantness response in contrast varies across type of sugar and subject populations (Sunday et al., 1992). The concentration range for sucrose from 0% to 32% in this study was associated with a linear slope of pleasantness in the past (Moskowitz, 1971), and thus we applied a linear statistical equation in order to calculate pleasantness slope for sucrose and sucralose (Splenda). We used the slope as a measure of pleasantness experience for the following reasons. Using the linear slope assesses the rate of change of pleasantness ratings as a function of sucrose concentration. This serves as a proxy measure for pleasantness sensitivity. This measure is more independent (1) from daily variations since it captures a response sensitivity rather than a single measure and (2) from

potential concentration differences in the batches of sucralose (Splenda) provided.

A second taste test, as preparation for the fMRI study component, determined for each individual a specific sucralose (Splenda) solution concentration that could not be distinguished from a 10% sucrose solution. Subjects were presented with 30 medicine cups, lined up as two-cup comparisons, each containing about 2 mL of sweet solution. Comparing two samples improves the ability to distinguish different tastes (Adolphs et al., 2005). Subjects reported which cup they believed was filled with sucrose and which one was filled with sucralose (Splenda) solution. The concentration of the sucralose (Splenda) solution was adjusted for each subject such that correct responses fell between 40% and 60% (12–18 samples), which indicated the subject's ability to distinguish the two solutions was at chance level. Sucralose (Splenda) solutions were initially made by adding 1.2 g of sucralose (Splenda) to 100 g of water. However, we needed between 1.25 and 1.30 g of sucralose (Splenda) across subjects in order to produce a solution that could not reliably be distinguished from the sucrose solution. Subjects were allowed to wash their mouth with distilled water between taste stimulations in order to avoid desensitization to or overload from the sweet tastes. The 10% sucrose solution and the individually found concentration of sucralose (Splenda; mean 1.26%) were subsequently used for the brain imaging study. Behavioral data were analyzed with SPSS14 statistical software (SPSS, Chicago, IL).

#### *Brain imaging procedures*

Functional magnet resonance imaging (fMRI) measuring blood oxygen level-dependent (BOLD) brain response was performed within the same (taste test) 10-day early follicular cycle episode, after the taste tests or during the early follicular cycle of the next cycle. On the day of the study, subjects were instructed to fast overnight and arrived at the fMRI facility between 7 and 8 AM. Subjects were given a standardized breakfast (bagel, cream cheese, banana, orange juice, skim milk, 604 total calories) with the instruction to “eat until feeling comfortably full, without overeating.” Subjects ate between 50% and 100% of the offered breakfast. Subjects were tested for potential pregnancy and screened for metal in their body before being placed in the fMRI scanner.

#### *Taste solution delivery*

One-milliliter fluid samples with sucrose or sucralose (Splenda) solution were delivered with a semi-automatic programmable customized syringe pump (J-Kem Scientific, St. Louis, MO). This design has been described previously (Frank et al., 2003). In brief, the syringe pump was located in the MRI-technician room with tubing running to the subject's mouth in the fMRI scanner. The tubes were fixated at the fMRI head holder, and end pieces of two silicon tubes were attached to each other and placed about 3/4 in. into the middle of the subjects' mouth. Subjects exerted no effort to hold the tubes in place, minimizing any sucking on the tubes. The syringe pump's hardware was connected to a laptop PC (Dell600, Dell, USA) in the technician room. This PC controlled the rate of administration of the solution (1 mL/s), the solution choice, and was also the interface between the syringe pump and the MRI scanner control panel. E-Prime software (Psychology Software Tools, Inc., Pittsburgh, PA) was used to coordinate taste stimulation with the fMRI scanning procedure.

Six blocks of taste stimulation were applied: two blocks with sucrose only, two blocks with sucralose (Splenda) only and two blocks with pseudorandom order of sucrose and sucralose (Splenda). Each block consisted of twenty 1-mL taste stimulations 20 s apart. The block order was fully randomized across subjects. During the taste stimulations, a total of 6 g sucrose was applied (24 kcal).

#### *Acquisition of images*

Imaging experiments were performed on a 3-T GE CXK4 Magnet. Each session consisted of a three-plane scout scan (10 s), a sagittally acquired spoiled gradient recalled (SPGR) sequence (field of view 25 cm; matrix: 194 × 256; 172 slices, thickness: 1.5 mm; TR: 20 ms; TE: 4.8 ms; and T2\* weighted echo-planar imaging (EPI) scans to measure blood oxygen level-dependent (BOLD) functional activity during taste stimulation (3.43 × 3.43 × 2.6 mm voxels, TR=2 s, TE=30 ms, flip angle of 90°, and 30 slices, with 2.6 mm slice thickness and a 1.4-mm gap). These parameters were used to minimize signal drop out close to air filled sinuses and to cover the entire brain.

#### *Image preprocessing pathway*

All image preprocessing and analysis were done with the Analysis of Functional Neuroimages (AFNI) software package (Cox, 1996). Preprocessed data were analyzed with a multiple regression model that used regressors derived from the experimental paradigm convolved with a prototypical hemodynamic response function (Boynton et al., 1996) to predict the echo planar signal change in response to either sucrose or sucralose (Splenda). In addition, five nuisance regressors were entered into the linear regression model: three movement-related regressors were used to account for residual motion (in the roll, pitch and yaw directions), and regressors for baseline and linear trends were used to eliminate slow signal drifts and to obtain baseline echo planar signal intensity. To account for individual variations in the anatomical landmarks, a Gaussian filter with full width at half maximum 6.0 mm was applied to the voxel-wise percent signal change data. All functional data were normalized to Talairach coordinates.

#### *Comparison of taste stimuli*

Voxel-wise percent signal change data for the whole brain were entered into unpaired *t*-tests for main effect BOLD response differences in sucrose and sucralose (Splenda) stimulations; data were also entered into paired *t*-tests for differences in BOLD response comparing sucrose and sucralose (Splenda) conditions. Main effect statistical maps were thresholded at  $p < 0.005$  and 1024  $\mu\text{L}$  (minimum of 16 contiguous voxels). Comparison maps subtracting sucrose from sucralose (Splenda) were thresholded at  $p < 0.05$  and 512  $\mu\text{L}$  (minimum of 8 contiguous voxels). In order to avoid confluent regions of interest, an anatomical mask was applied on the statistical maps to retrieve activity data related to the taste pathway: midbrain, thalamus, caudate, putamen, anteroventral striatum, insula, anterior cingulate and orbitofrontal cortex. Percent signal change data were extracted from regions of interest (ROIs) that survived this thresholding, clustering and masking protocol. Those percent signal change data were also used to assess correlation or regression analyses with behavioral data defined in the hypotheses.

### Taste pathway analysis, functional connectivity analysis

The FO/AI as the primary taste cortex was selected as seed regions in order to assess taste pathway-related areas. We selected the main effect statistical results for sucrose and sucralose (Splenda) in the FO/AI as seed regions. Two analyses were conducted. First, a simple connectivity analysis that assessed brain areas that are temporally and thus functionally related (general taste pathway). Second, we assessed functional connectivity in relation to pleasantness ratings from the behavioral assessments (taste pathway activation specific to pleasantness experience).

Before conducting the functional connectivity analysis, echo planar signals were corrected for slice-dependent time shifts, spatially filtered using a 6-mm FWHM Gaussian filter and temporally filtered using a bandwidth filter ( $0.009 < f < 0.08$ ). The resulting echo planar time series was then warped to Talairach space.

Individual time courses were extracted from these processed echo planar signals for a seed ROI that showed task-dependent activation, specifically the bilateral FO/AI. Correlations due to possible head motion were eliminated by censoring time points that were more than 2 SD from the individual's average activation for the given seed ROI. The extracted time courses were smoothed, by applying a 0.5 weight to the current slice and 0.25 weight to the time point immediately before and after, and deconvolved as the 5 regressors of interest, along with the 5 nuisance regressors described above. Voxel-wise correlation coefficients were Fisher  $Z$  transformed. A paired  $t$ -test was performed for each seed ROI that contrasted the Fisher  $Z$  transforms of the correlation coefficient in the sucrose and sucralose (Splenda) conditions, so as to determine differences in functional connectivity between conditions. A voxel-wise regression analysis was used to correlate functional connectivity data with the pleasantness slope for each subject.

## Results

### Taste behavioral measures

As expected, sweetness ratings correlated highly with concentration for sucrose (Pearson correlation mean  $r=0.9$ ,  $p<0.0001$ , range from  $r=0.86$ ,  $p=0.03$  to  $r=0.99$ ,  $p<0.0001$ ) as well as sweetness and concentration for sucralose (Splenda) (Pearson correlation mean  $r=0.8$ ,  $p<0.0001$ , range from  $r=0.5$ ,  $p=0.3$  to  $r=0.99$ ,  $p=0.0001$ ). Pleasantness relation with sweetness was more variable for either sucrose (Pearson correlation mean  $r=-0.09$ ,  $p=0.4$ , ranging from  $r=-0.97$ ,  $p=0.002$  to  $r=0.96$ ,  $p=0.002$ ) or sucralose (Splenda) (Pearson correlation mean  $r=0.03$ ,  $p=0.8$ , ranging from  $r=-0.76$ ,  $p=0.07$ , to  $r=0.97$ ,  $p=0.001$ ). We then calculated the individual subject slopes for the pleasantness measure. This resulted in slopes ranging for sucrose from  $-0.25$  to  $0.28$  and for sucralose (Splenda) from  $-0.24$  to  $0.2$ . The pleasantness slopes for sucrose and sucralose (Splenda) showed a very high correlation (Pearson  $r=0.8$ ,  $p=0.001$ ; Fig. 1).

### fMRI results

Due to the limited detection power of the pseudorandomized presentation of sucrose or sucralose (Splenda) solutions, we only show the block application with repeated same stimulus application.

### Main effect, significance threshold $p=0.005$ , 16 voxel contiguity

At this threshold, the main effect of sucrose stimulation revealed bilateral activation in the FO/AI (extending from the frontal operculum to the inferior insula), left ventral striatum, anterior cingulate and bilateral midbrain. Sucralose (Splenda) stimulation also activated the FO/AI bilaterally to a similar extent when compared to sucrose-related activation, but no other area. In addition, both sucrose and sucralose (Splenda) activated bilateral sensorimotor cortical areas. Within the anatomical regions of interest, overlaid on the statistical maps, sucrose was found to induce significant activation in 10 regions. In comparison, sucralose (Splenda) administration resulted in significant activations in only 3 of the a priori determined regions (Table 1). Fig. 2 contains the main effect activation map for each sweet taste condition.

### Main effect data correlation with behavioral data

In order to relate subjective assessments of pleasantness with fMRI percent signal change, a regression analysis was conducted with pleasantness ratings as the independent variable and signal change as the dependent measure. The FO/AI activation was selected as imaging variable since it is the primary taste cortex and that area was activated similarly across conditions. This analysis (Fig. 2) showed that pleasantness slopes predicted left insula activation for both sucrose ( $p=0.01$ ) and sucralose (Splenda) ( $p=0.03$ ) conditions. In comparison, there was only a tendency for pleasantness slopes to predict right-sided insula activation for both sucrose ( $F=2.7$ ,  $p=0.1$ ) and sucralose (Splenda) ( $F=2.6$ ,  $p=0.1$ ) administration.

### Sucrose versus sucralose (Splenda) comparison

A direct comparison of activation due to sucrose or sucralose (Splenda) administration revealed greater activation for sucrose in the bilateral FO/AI; other ROIs were localized to the left caudate, left cingulate and bilateral superior frontal cortex, in addition to the posterior part of the anterior insula bilaterally (Fig. 3, Table 1).

### Functional connectivity

Three analyses were conducted.

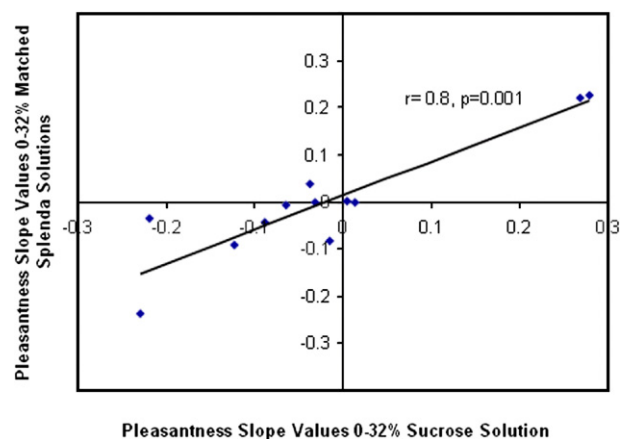


Fig. 1. Subjects pleasantness slope correlation for sucrose and sucralose.

Table 1

sucrose and Splenda statistical results as regions of interest (ROIs) for sucrose and sucralose condition main effects, as well as for subtraction sucrose–sucralose results

	Volume ( $\mu\text{L}$ )	<i>x</i>	<i>y</i>	<i>z</i>	Description
<i>Sucrose main effect regions of interest</i>					
1	4672	−39	0	5	Left frontal operculum, anterior insula, claustrum
2	4608	39	−3	9	Right frontal operculum, anterior insula, claustrum
3	4160	0	6	38	Bilateral mid-sagittal cingulate gyrus, BA24 and BA32
4	1280	28	45	28	Right superior frontal gyrus: BA9 and BA10
5	1152	−14	10	−2	Left anteroventral striatum with nucleus accumbens
6	512	6	−15	10	Right thalamus, medial dorsal nucleus
7	448	−11	−20	−6	Left midbrain: substantia nigra, VTA
8	320	44	−15	3	Right temporal operculum
9	320	−14	6	18	Left dorsal caudate
10	256	10	−21	−7	Right midbrain: substantia nigra, ventral tegmental area
<i>Splenda main effect regions of interest</i>					
11	3584	38	−2	7	Right frontal operculum, anterior insula, claustrum
12	2688	−40	−3	5	Left frontal operculum, anterior insula, claustrum
13	576	29	45	28	Right superior frontal gyrus: BA9 and BA10
<i>Sucrose minus Splenda regions of interest</i>					
14	1216	−35	17	5	Left frontal operculum, anterior part of anterior dorsal insula
15	896	−39	3	12	Left posterior part of anterior insula
16	896	2	7	58	Bilateral superior frontal gyrus, BA6
17	832	38	−1	13	Right posterior part of anterior insula
18	640	−9	12	6	Left middle caudate
19	640	−20	48	21	Left superior frontal gyrus, BA10
20	448	−11	5	36	Left anterodorsal cingulate, BA24
21	320	−15	38	12	Left anterior cingulate, BA32
22	256	−6	13	40	Left anterodorsal cingulate, BA32

First, to delineate the circuitry associated with administration of both sucrose and sucralose (Splenda), we examined the functional connectivity between the activated insular cortex and other brain areas. The two insula ROIs (ROIs 1 and 2; Table 1) were determined based on the main effect of sucrose and sucralose (Splenda) within this structure (Fig. 4a). Overall, there was a similar pattern of connectivity for sucrose and sucralose (Splenda), with seed regions in the left FO/AI being correlated with activation in the contralateral insula, ipsilateral ventral and middle striatum and pallidum, bilateral anterior cingulate and ipsilateral thalamus. The right FO/AI (ROI 2 for sucrose and ROI 11 for sucralose (Splenda)) activation was correlated with contralateral insula, bilateral anterior cingulate and bilateral thalamus; no correlation of activity was found for the striatum. Second, to determine whether there was a significant difference in the degree of functional connectivity between the areas co-activating in response to sucrose relative to sucralose (Splenda), we conducted a voxel-wise paired *t*-test compared Fisher *Z*-transformed *R* values from the functional connectivity analysis in sucrose and sucralose (Splenda) conditions. Data were clustered at  $p=0.05$  and 512  $\mu\text{L}$  (8 contiguous voxels) showing higher connectivity in the sucralose (Splenda) condition compared to sucrose for both ROIs 1 and 2 for sucrose and ROIs 11 and 12 for sucralose (Splenda) (Fig. 4b).

Third, to assess if there are brain areas connected to the FO/AI that specifically mediate the pleasantness sensitivity, we applied a regression analysis using voxel-wise  $R^2$  values from the functional connectivity analysis and the pleasantness slope values. This showed that, for the sucrose condition, pleasantness slopes predicted connectivity of ROI 1 with the contralateral insula and midbrain, including the substantia nigra/VTA (Fig. 4c). These

findings were absent in the sucralose (Splenda) condition. Pleasantness slopes predicted connectivity of ROI 2 with the contralateral insula in the sucrose condition; these findings were also absent in the sucralose (Splenda) condition.

## Discussion

This study indicates that caloric sucrose relative to sucralose (Splenda) activates more strongly taste pathway regions such as the FO/AI, striatum, anterior cingulate and prefrontal cortex. Although FO/AI activation by both sucrose and sucralose recruited similar taste processing-related areas, sucralose (Splenda) showed a stronger connectivity between those regions than sucrose. Pleasantness slopes for both sweet stimuli predicted significant left insula activation; however, only sucrose activation, related to the pleasantness ratings, engaged the contra lateral insula and midbrain, including the VTA and substantia nigra. This may indicate that sucrose is able to cause a different physiological brain response compared to sucralose with recruitment of more food reward-related brain regions, despite the inability of subjects to consciously distinguish the tastes.

## Behavioral results

Consistent with previous behavioral studies (Moskowitz, 1971), our behavioral data indicated that all subjects rated greater sweetener concentration with greater experienced sweetness. In contrast, some individual rated the sweeter stimuli more pleasant while others gave higher sweetness lower pleasantness ratings, indicating modest positive and negative responsivity across the

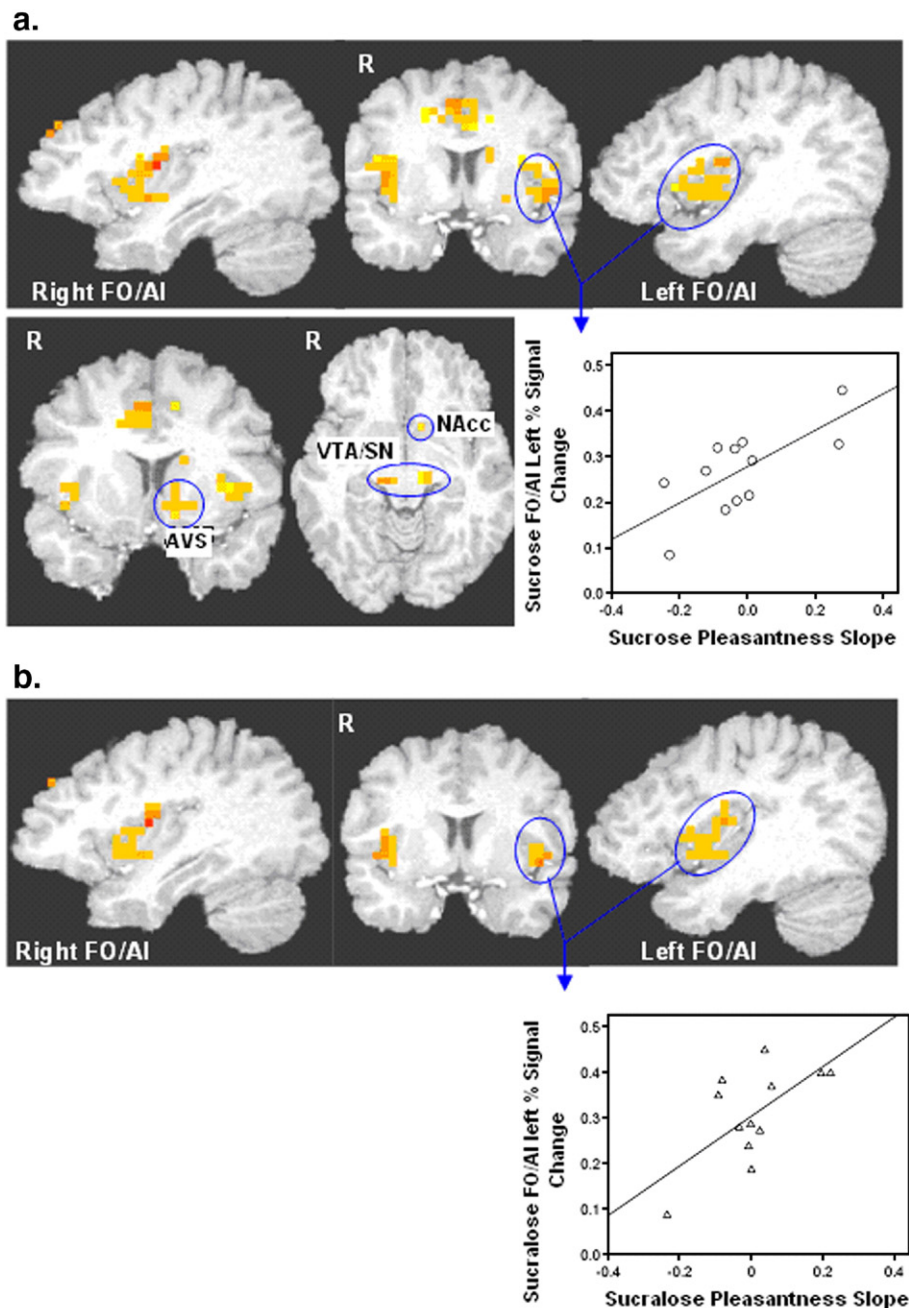


Fig. 2. Main effect activation maps (sampled at  $p=0.005$ , 16 voxel contiguity) for sucrose (a) and sucralose (b). Both sucrose and sucralose activate the bilateral primary taste cortex (frontal operculum, anterior insula, FO/AI). Only sucrose activated anteroventral striatum (AVS), nucleus accumbens (NAcc) and midbrain substantia nigra and ventral tegmental areas (SN/VTA). Blue arrows point to regression line graphs for left FO/AI percent signal change with sucrose ( $p=0.01$ ) and sucralose ( $p=0.025$ ) pleasantness slope ratings.

stimulations. Such inter-individual differences for the hedonic experience of sucrose solutions across a concentration gradient has been reported previously (Prescott et al., 1992). We calculated the slope for the individual sweetness ratings in order to capture intra-subject change of behavioral response between the different sweet stimulus concentrations. Slopes for sweetness and pleasantness response have been used in food appraisal research in the past (Moskowitz, 2001) and indicate a responsiveness across a range of stimulation strengths. The slopes for sucrose and sucralose

(Splenda) solution responses were highly correlated with each other, suggesting that the hedonic sweet taste responsivity as a measure of “liking,” was quite similar across conditions.

#### fMRI activation results

The human primary gustatory cortex lies within the FO/AI (Pritchard et al., 1986; Yaxley et al., 1990). This region is defined by the area that receives direct projections from the parvocellular

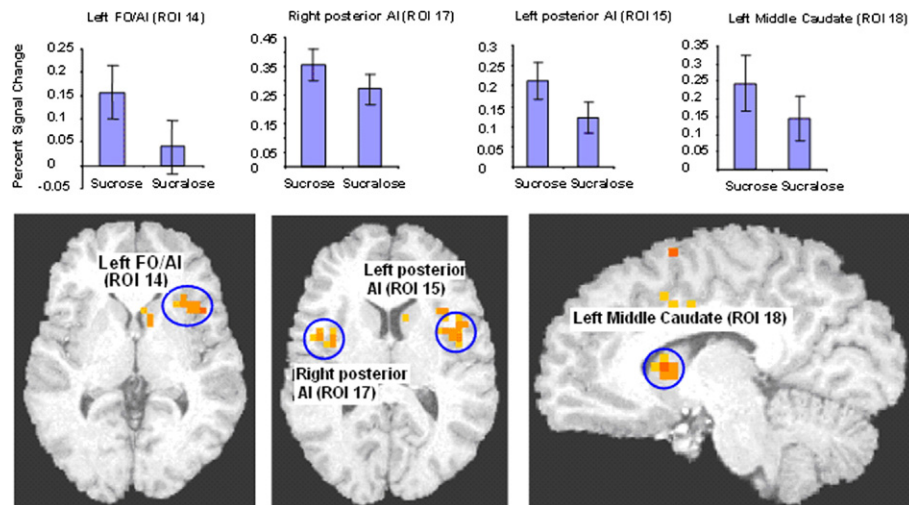


Fig. 3. Subtraction maps ( $p=0.05$ , 8 voxels contiguity) and associated blood oxygen level-dependent signal expressed as percent signal change for sucrose minus sucralose. Blue circles indicate selected regions of interest (ROI). FO/AI, frontal operculum/anterior insula; for ROI numbers, refer to Table 1.

portion of the ventroposterior medial nucleus of the thalamus (VPM), the thalamic taste nucleus. Gustation has been recognized as a rather complex process with inputs from the tongue taste receptors, brain stem, thalamus and limbic system (Jones et al., 2006) that involves a complex interplay of neurotransmitters at cortical and subcortical synapses. Projections from the primary taste cortex reach the central nucleus of the amygdala, and from there, the lateral hypothalamus and midbrain dopaminergic regions (Simon et al., 2006). The primary taste cortex also projects heavily to the striatum (Chikama et al., 1997; Fudge et al., 2005). The posteromedial, lateral and posterolateral agranular insula, which processes gustatory, olfactory and internal organ information (Carmichael and Price, 1996), has the strongest connections to the ventral striatum (Fudge et al., 2005). The anterior insula is contiguous with the posterior orbitofrontal cortex at the operculum. This region is reciprocally connected with the medial prefrontal cortex which includes the anterior cingulate (Carmichael and Price, 1996). Functionally, neuroimaging studies have shown that both pleasant and unpleasant stimuli activate the orbitofrontal (O'Doherty et al., 2000), as well as the cingulate cortex (Rolls et al., 2003). The ventral striatum receives input from the FO/AI and anterior cingulate (Carmichael and Price, 1996; Fudge et al., 2005; Haber et al., 1995). It is associated with immediate reward processing (Tanaka et al., 2004) and prediction of future rewards (O'Doherty, 2004), while the anterior cingulate processes error monitoring and anticipation of reward (Carter et al., 1998; Richmond et al., 2003). Furthermore, a regional segregation of intensity (cerebellum, pons, middle insula and amygdala) and valence (FO/AI, orbitofrontal cortex) of gustatory stimuli has been reported (Small et al., 2003).

Both sucrose and sucralose (Splenda) main effects activated the FO/AI. However, sucrose also activated the left ventral striatum, left dorsal caudate nucleus, bilateral midbrain and right thalamus (in the region of the taste center, the ventromedial posterior nucleus), which are established components of taste reward circuits. Thus, sucrose activated taste pathway areas that sucralose (Splenda) did not activate at the set significance threshold. Of particular significance is the lack of midbrain involvement in the sucralose (Splenda) condition (see Discussion).

Our notion that sucrose relative to sucralose (Splenda) elicits greater absolute brain response in the taste pathway and downstream reward system is further supported by the direct comparison of the main effects on the brain areas important for taste and reward processing. Sucrose response was greater than sucralose (Splenda)-related activation of bilateral primary gustatory cortex, left middle striatum, left anterior cingulate and prefrontal cortex. One mechanism for this difference in brain activation across the two taste stimuli could be that sucrose acts on more than just the T1R3 taste receptors, compared to sucralose (Splenda) (Damak et al., 2003). An additional explanation could be a learning component. What we like to eat is genetically and environmentally influenced (Sclafani, 2006). Since infants are fed with caloric sugars and usually not with artificial sweeteners, it is possible that human feeding-related brain pathways are also conditioned to react stronger to the known, caloric stimulus. Another possibility is that the brain senses blood glucose levels (Levin, 2006) and for that reason responds stronger to sucrose, a response that may be particularly strong in the hunger state (de Araujo et al., 2006). In our study, we fed the participants in order to avoid the confound of the hungry state-related mild hypoglycemia responding greater to sugar. Taken together, these results indicate that behaviorally matched sucralose (Splenda) is not able to activate brain taste pathways similarly compared to sucrose. The cause for this difference needs further study.

This analysis also indicated a lateralization with particularly left-sided brain response greater after sucrose compared to sucralose (Splenda). Previously, a hemispheric asymmetry has been proposed with left-sided processing of parasympathetic activity, nourishment and appetitive behavior (Craig, 2005; Wittling and Roschmann, 1993). Our finding might support this view with greater left than right-sided brain activation in response to basic appetitive and pleasant food stimuli.

It is important to note that taste and reward processing are a result of a coordinated interplay between several different brain areas. Therefore, functional connectivity analyses between the primary taste processing area and other taste and reward processing regions are critical (Fudge et al., 2005) to determine whether sucrose and sucralose (Splenda) activations result in different taste

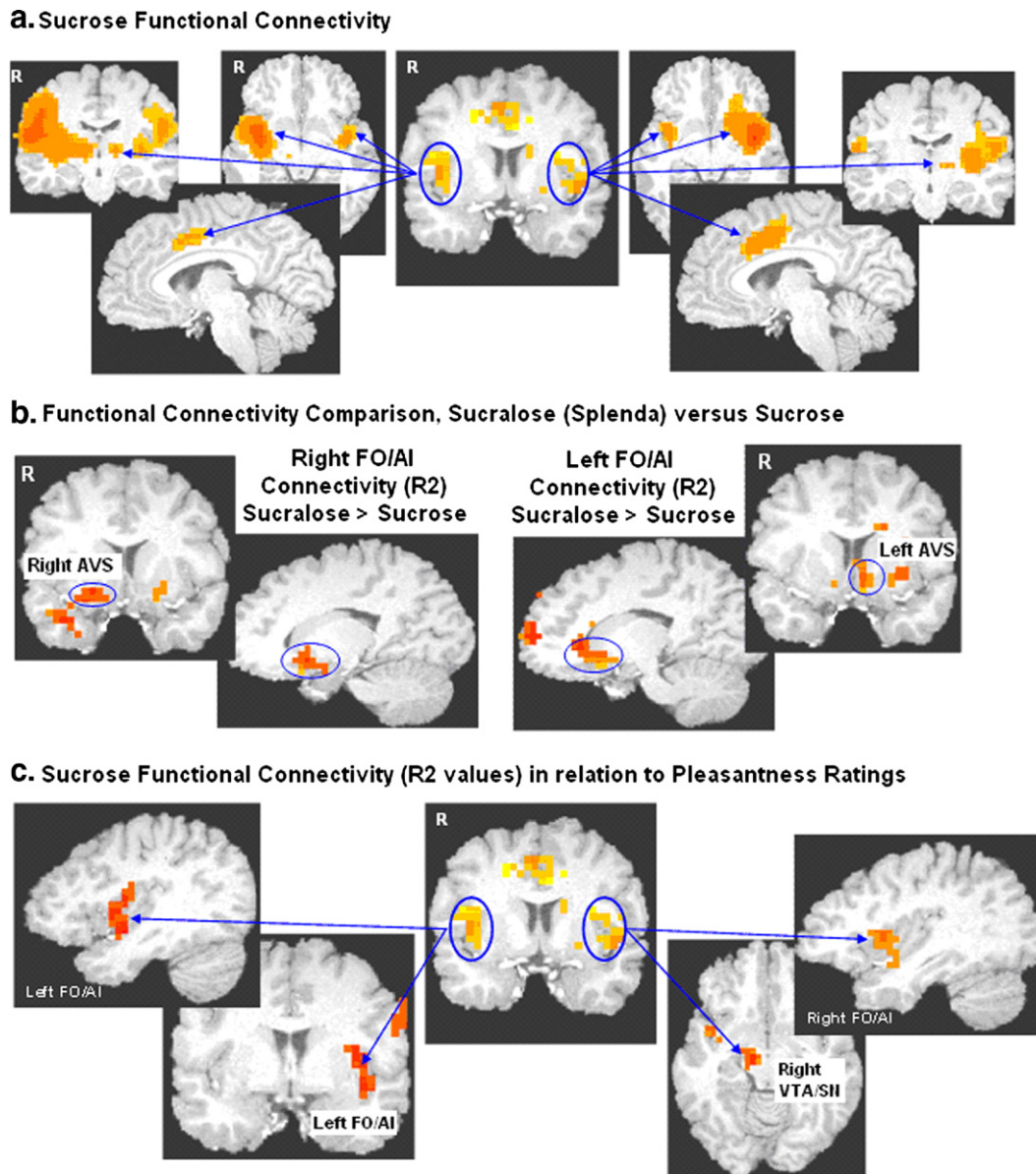


Fig. 4. (a) Functional connectivity analysis for sucrose, with the left and right frontal operculum/anterior insula (FO/AI) as seed regions. Insula activation is functionally connected to bilateral insula, cingulate and thalamus activation. Sucralose (Splenda) stimulation showed a similar pattern of activation (data not shown). (b) The subtraction of functional connectivity  $R^2$  values (insula as seed region) shows greater connectivity for sucralose (Splenda) compared to sucrose for both insula seed regions for the ipsilateral anteroventral striatum. (c) Connectivity  $R^2$  values in relation to the sucrose pleasantness ratings; both insula seed regions activate the contralateral insula: left Insula connectivity in relation with pleasantness is functionally also connected to the right ventral tegmental/substantia nigra (VTA/SN). In contrast, sucralose (Splenda) connectivity in relation to pleasantness ratings did not show significantly related brain activation.

and reward experiences. Our primary functional connectivity results for sucrose and sucralose (Splenda) brain activation are consistent with those anatomical pathways of taste processing (Chikama et al., 1997; Fudge et al., 2005; Pritchard et al., 1986). Although the largely similar patterns of taste-related brain activation, there were several notable differences. When we subtracted sucralose (Splenda) from sucrose connectivity, i.e. the strength of connectivity ( $R^2$  values) with related brain regions, using the left and right insula as seed regions, we found that sucralose (Splenda) showed a stronger connectivity compared to sucrose. This result was surprising because it may indicate that,

while the absolute brain response after sucralose (Splenda) is lower than for sucrose, the recruitment of related brain regions is stronger. The implication of this result is uncertain. However, it raises several questions: If a sweet taste without caloric value turns on the taste reward circuitry with higher “functional connectivity” which can be interpreted as more synchronized response – potentially mediated by sucralose high affinity (Nie et al., 2005) for only the T1R3 receptor (Damak et al., 2003) while sucrose may act via additional receptor input – but with lesser strength compared to sucralose (Splenda), how does this affect the feedback mechanisms in terms of “wanting” more of that stimulus? Higher



sucrose concentrations are anecdotally usually described as syrup like and less pleasant. This sensation may be a safeguard in order not to consume too much sugar. If sucralose (Splenda) activates the taste pathways without such a safeguard, one wonders if there is still a need for satisfying a “sweet tooth” or aspect of internal negative feedback. Sweet taste receptors that are specific for caloric carbohydrates including sucrose (Damak et al., 2003) could be responsible for such a mechanism.

One of our questions and hypotheses was whether there are biologic mechanisms that code the “liking” or hedonic response, even in the absence of conscious distinction of sweet stimuli or the acute rating. The slope of pleasantness response across various concentrations provides a measure of responsivity that is independent from an absolute and somewhat arbitrary rating scale. We thought that the slope for individual pleasantness ratings across many concentrations would capture the disposition to respond with the fMRI BOLD measure. In other words, while the behavioral pleasantness ratings and associated slopes indicated how sensitively someone gets “turned on” by the sweet stimuli, the brain region that corresponds to this measure then might be the area that regulates this process. Since the anterior insula was activated for both sucrose and sucralose (Splenda) main effects, we assessed those ROIs in relation to the pleasantness ratings. For both sucrose and sucralose (Splenda), a regression analysis indicated that pleasantness slope predicted left-sided FO/AI brain response for sucrose and sucralose (Splenda) individually. This result supports a previous finding (Wagner et al., *in press*) that the insula takes part in the brain hedonic response to taste stimuli. It is possible that the insula sets the tone for how strong of a signal is transmitted to areas that further process hedonic taste experience.

Furthermore, we computed a new set of connectivity analyses that investigated regional brain activation that is temporally related to the left FO/AI activation and related to the pleasantness slopes. This analysis suggested that the sucrose but not the sucralose (Splenda) condition activates the midbrain and contra lateral insula concurrent with the left anterior insula. Right-sided sucrose, but not sucralose (Splenda), FO/AI activation was related to contralateral insula activation in conjunction with the pleasantness rating. An exploratory reduction of the significance threshold did not show midbrain or contralateral insula connectivity with the sucralose (Splenda) activated left FO/AI.

Indeed, these data may indicate that sucrose, in relation to hedonic experience, specifically recruits distinct pathways compared to sucralose (Splenda), areas that are known to be related to reward processing (O'Doherty et al., 2003; Schultz, 2002).

#### *Exploratory post hoc analysis*

Recent data using a tasteless solution (Veldhuizen et al., 2007) indicate that the process of detecting a taste may in particular recruit the middle dorsal insula together with the middle frontal gyrus/frontal eye field, Brodmann area (BA) 32 of the dorsal anterior cingulate, the parietal operculum, the thalamus, the substantia nigra and the bilateral cerebellar areas. Our study did not involve an explicit taste detection task, but from self report we know that study participants do try to detect which sweet taste they are receiving. While main effect activation for both sucrose and sucralose (Splenda) recruited a cluster of activation covering large parts of the anterior insula, the insula high intensity voxels (areas of highest activation) were in fact in the middle insula, over the

middle and posterior short gyrus of the right (sucrose and sucralose:  $x=37, y=-9, z=12$ ) and left (sucrose:  $x=-35, y=-9, z=11$ ; sucralose:  $x=-36, y=-10, z=11$ ) anterior insula. Thus, in an exploratory analysis, we assessed if we would find similar areas of associated activation as others (Veldhuizen et al., 2007) when receiving ambiguous tastes. We created an anatomical mask that included only the middle insula, restricted the statistically derived main effect activation maps to that mask, used those left and right middle insula regions as seed region for an additional connectivity analysis and subtracted sucrose from sucralose (Splenda) connectivity.

The right insula seed region resulted in co-activation that was always greater for sucrose compared to sucralose (Splenda) in the left anterior cingulate ( $x=-3, y=47, z=8$ ), left middle cingulate ( $x=-9, y=-19, z=32$ , BA24/23, right medial frontal cortex ( $x=7, y=40, z=28$ ), bilateral occipital cortex ( $x=-20, y=-72, z=17$ ;  $x=15, y=-62, z=14$ ), right pregenual cingulate ( $x=-3, y=47, z=8$ ) and bilateral midbrain including the left substantia nigra ( $x=-6, y=-13, z=-10$ ). The comparison of left insula seed region connectivity between the two tastes resulted in greater activation for sucralose (Splenda) versus sucrose in the left superior frontal gyrus/orbitofrontal cortex ( $x=-29, y=56, z=-4$ ), right inferior parietal lobule and BA40 ( $x=46, y=-43, z=43$ ), left rostral cingulate and right angular gyrus ( $x=39, y=-65, z=33$ ), but greater activation for sucrose versus sucralose (Splenda) in the right superior frontal gyrus ( $x=6, y=30, z=49$ , frontal eye field/BA6 and BA8), the right middle frontal gyrus ( $x=25, y=18, 45$ , BA6 and BA8) and the left and right posterior cingulate ( $x=-2, y=-43, z=25$ ). The results from this analysis using the left and right middle insula as seed regions for connectivity analysis may support the findings of Veldhuizen et al. (2007) that this area, together with frontal cortical regions including the frontal eye field, cingulate cortex and midbrain are likely involved in cognitive processes of taste detection. Our study was not designed to explicitly test taste detection since we tried to focus on unconscious taste detection, and the results from this post hoc analysis have to be viewed with caution. It can also not be easily explained why sucrose and sucralose (Splenda) have varying greater or lesser connectivity activation.

#### *Study design considerations*

In this paradigm, we applied taste stimuli repeatedly within blocks of activation, although subjects were not aware of what taste stimulus they received. This is important since we attempted to avoid cognitive bias as well as the effect of novelty. Thus, we could only expect to see brain activation in areas that are known to respond to taste stimuli repeatedly. Parts of the insula respond distinctly to different taste qualities (Yaxley et al., 1990), and food-related insula stimulation is continuous, not appetite dependent (Scott and Plata-Salaman, 1999). The ventral striatum has also been shown to respond to repeated taste stimulation without loss of responsiveness (Mora et al., 1979). The orbitofrontal cortex in contrast loses activation when the same stimulus is repeatedly presented (sensory-specific satiety). We did not find significant orbitofrontal cortex activation. In addition, our subjects were fed prior to the study and this further may reduce orbitofrontal cortex activity. The lack of orbitofrontal cortex activation was not due to signal drop out.

fMRI does not directly assess neurotransmitter function, and we did not apply a challenge drug in this study. However, dopamine

(DA) and opioid neurotransmitters have been most consistently related to taste reward. The midbrain and ventral striatum DA neurons respond to unexpected positive or negative reward with activation or deactivation (O'Doherty et al., 2003), probably related to the motivational aspects of reward or “wanting” (Kelley et al., 2005). Pleasantness, the “liking” or hedonic response in the food reward pathway, may be mostly related to opioid activation (Kelley et al., 2005). The DA and opioid neurons and receptor types are colocalized in the ventral striatum and probably also in the midbrain, and interactions of the two systems on the level of the ventral tegmental area have been reported (Stinus et al., 1980). Thus, it is speculative what particular neurotransmitter system may be involved in this experiment. We do, however, believe that opioid neurotransmission may well be mediating the brain response that is related to the pleasantness response. In fact, sweet taste enhances opioid release, which in turn increases the palatability of sweet taste (Yamamoto, 2003).

### Limitations

This study recruited women only and the response patterns may be different in men. This will need to be tested. Gender differences for sweet taste perception have been reported (Curtis et al., 2004), and hormonal changes during the female reproductive cycle affect brain response such as reward pathways (Dreher et al., 2007). Thus, this study focused on women in the early follicular cycle, where there is relative hormonal stability, in order to minimize potentially confounding effects.

We could have placed anatomical seed ROIs and assessed brain activation on an ROI basis as well as use anatomical ROIs for the connectivity analysis. We decided against this method since there are inter-individual taste responses, and we felt that using statistical ROIs for correlation and regression analyses would reveal the most reliable results. We made every effort to keep confounding circumstances, including having subjects receive a standard breakfast with the instruction to eat until comfortably full. This probably contributed to no significant orbitofrontal cortex activation, and with the study design of repeated stimulus application, sensory-specific satiety has to be expected with a loss of orbitofrontal response. There was no rinse between taste stimulations in the fMRI scanner. However, on the day of behavioral testing, there was water provided to rinse in between taste testing. Since we present only the blocks of repeated stimulus application, we believe that the fMRI signal is related to the taste stimulus reported and not diluted by the alternate sweet taste. Sucrose could have altered gut response and blood glucose, while sucralose (Splenda) could not and this could have contributed to altered brain response. However, the total amount of sucrose applied during the entire experiment was 6 g or 24 kcal and thus small in comparison to the standard breakfast that was eaten to 50% or more across all subjects. It is therefore unlikely that this small amount of sucrose would have altered blood glucose and brain response in a meaningful way. While sucralose is the active ingredient in Splenda, there is a small amount of maltodextrin added for bulk. The total calorie amount of maltodextrin is considered negligible; however, the taste response to maltodextrin could have influenced the results. We think that using the commercially available sucralose (Splenda) is valid for the following reasons. First, in order to assess real-life relevant behavior, it is most appropriate to use compounds available to the public. Second, if anything, the maltodextrin should have shifted the results toward the

sucrose response and thus not account for the sucralose (Splenda)–sucrose differences.

### Implications

There are various potential implications that can be raised from the results of this study. It is not resolved yet if the long-term use of artificial sweeteners truly leads to reduced body weight. If it holds, that caloric sucrose stimulates taste reward brain regions more, but that sucralose (Splenda)-activated brain regions are more connected, one wonders if sucralose (Splenda) stimulates the food reward system faster or more efficiently. Since sucralose (Splenda) does not provide calories and thus no natural feedback mechanism of biologic satiety, it is possible that this lack of feeling of satiety has to be met with other – probably caloric – means and therefore potentially defeating the purpose of sucralose (Splenda) use.

Second, the anterior insula is here once again highlighted as a sensory but also hedonic emotional integration area. If it is true that the insula sets the tone for downstream food-pleasantness-related reward activation, then this area, and in particular the left-sided FO/AI, may be a target for food reward modulation.

### Summary

Taken together, this study indicates that sucrose and sucralose (Splenda) activate common taste pathways, but the primary taste cortex as well as pleasantness-related brain reward circuitry are activated greater for sucrose compared to sucralose (Splenda). In contrast, sucralose (Splenda) activation recruits a stronger connectivity between taste pathway regions without reaching the magnitude of brain response for sucrose. This may suggest that sucralose (Splenda) activates taste reward circuits but may not fully satisfy a desire for natural caloric sweet ingestion.

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