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The Representation of Oral Fat Texture in the Human Somatosensory Cortex

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Abstract: How fat is sensed in the mouth and represented in the brain is important in relation to the pleasantness of food, appetite control, and the design of foods that reproduce the mouthfeel of fat yet have low energy content. We show that the human somatosensory cortex (SSC) is involved in oral fat processing via functional coupling to the orbitofrontal cortex (OFC), where the pleasantness of fat texture is represented. Using functional MRI, we found that activity in SSC was more strongly correlated with the OFC during the consumption of a high fat food with a pleasant (vanilla) flavor compared to a low fat food with the same flavor. This effect was not found in control analyses using high fat foods with a less pleasant flavor or pleasant-flavored low fat foods. SSC activity correlated with subjective ratings of fattiness, but not of texture pleasantness or flavor pleasantness, indicating a representation that is not involved in hedonic processing per se. Across subjects, the magnitude of OFC-SSC coupling explained inter-individual variation in texture pleasantness evaluations. These findings extend known SSC functions to a specific role in the processing of pleasant-flavored oral fat, and identify a neural mechanism potentially important in appetite, overeating, and obesity. *Hum Brain Mapp 35:2521–2530, 2014.*

Key words: reward; fMRI; taste; flavor; food; orbitofrontal cortex

INTRODUCTION

The sensory properties of food and their hedonic effects are important drivers of food intake, and oversensitivity of the brain's reward system to these sensory properties may be a key factor in obesity [Rolls, 2005, 2007, 2012b, 2014; Stice et al., 2008; Wardle et al., 2001]. One of the sensory properties of food important in making it palatable is its fat texture or mouthfeel [Drewnowski, 1998]. Investigations to examine the representation of oral fat in the brain to understand the principles of its neural processing showed that at the neuronal level there is a representation of fat in the mouth in the primary taste cortex, orbitofrontal cortex (OFC), and amygdala [Kadohisa et al., 2005b; Rolls, 2011a; Rolls et al., 1999; Verhagen et al., 2003, 2004]. These neurons responded on the basis of the oral texture of fat, and some evidence was found that the pleasantness of fat texture was represented in the primate OFC [Rolls, 2011a; Rolls et al., 1999; Verhagen et al., 2003]. We have shown in a human fMRI investigation that oral fat texture is represented in areas of the human brain such as the taste and somatosensory insula, OFC, and anterior cingulate cortex [de Araujo and Rolls, 2004]. Recently, we showed that neural activity in the human mid-orbitofrontal and anterior cingulate cortex was correlated with the pleasantness of

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oral fat texture, and in nearby locations with the pleasantness of flavor [Grabenhorst et al., 2010].

In this investigation, to understand more about the neural representation of oral fat and its pleasantness, we build on the preceding investigation [Grabenhorst et al., 2010] and examine, using psychophysiological interaction (PPI) analysis [Friston et al., 1997; Gitelman et al., 2003], which regions of the brain are functionally connected to the OFC during the consumption of pleasant high fat foods. Oral fat is particularly pleasant when combined with consonant flavors as in vanilla ice cream [Drewnowski, 1995]. Although obese individuals do not necessarily report stronger hedonic responses to high fat foods, increased liking for such foods can predict increased food intake and weight gain [Bartoshuk et al., 2006; Salbe et al., 2004]. Therefore, we searched for brain regions in which functional coupling with OFC occurred specifically for oral fat in combination with a pleasant vanilla flavor, but not for oral fat in combination with a less pleasant flavor or for pleasant-flavored stimuli that were low in fat. Understanding the representations in the human brain of oral fat [de Araujo and Rolls, 2004; Eldeghaidy et al., 2011a], and its pleasantness [Grabenhorst et al., 2010], may help unravel possible differences in how neural reward systems in obese versus lean people respond to oral fat, a driver of food intake, and in the design of foods that produce the mouthfeel of fat yet have low energy content.

METHODS

Design

The flavor stimuli consisted of a pleasant vanilla-flavored dairy drink and, to provide for a range of pleasantness ratings in the investigation, a less pleasant strawberry-flavored dairy drink. Both types of flavor stimuli were presented as a low fat version (0.1% fat milk) and a high fat version (single cream, 18% fat) to produce a range of liquid food stimuli that differed in taste, olfactory, and texture components. The drinks were made by taking either single cream or the low fat milk as the base, and the flavor component was specified by vanilla food flavor and 5 g/100 ml (0.15 M) sucrose, or by strawberry food flavor without sucrose. The design thus provided for two levels of fattiness, and two levels of the pleasantness of the flavor. The strawberry flavor was selected based on pre-testing in an independent subject sample. In these pre-tests, we found that the particular strawberry flavor used was rated as less pleasant than the vanilla flavor. We, therefore, used it in this study to vary flavor pleasantness as the strawberry flavor provided a good match to the vanilla flavor with respect to the semantic congruity between fat-flavor combinations. The tasteless control/rinse solution contained the main ionic components of saliva (25 mM KCl + 2.5 mM $NaHCO_3$) which when subtracted from the effects produced by the taste stimulus allowed taste (or in the case of the high fat stimuli taste and fat texture) effects to be distinguished from general somatosensory effects produced by introducing fluid into the mouth and any mouth movement [de Araujo et al., 2003a; O'Doherty et al., 2001]. Flavor stimuli were delivered to the subject's mouth through teflon tubes (one for each of the 4 stimuli, and a separate tube for the tasteless rinse control) that were held between the lips. Each teflon tube of approximately 3 m in length was connected to a separate reservoir via a syringe and a one-way syringe activated check valve (Model 14044-5, World Precision Instruments), which allowed 0.75 ml of any stimulus to be delivered at the time indicated by the computer.

The trial structure is shown in Figure 1A. Each trial was preceded by a visual cue displayed for 1 s to indicate to the subjects that a flavor stimulus will be delivered. At trial start, a blue cross was shown and a flavor stimulus (chosen in a random permuted sequence through the set of stimuli that was then followed by a new random permutation) was delivered into the mouth, and left there for a 7 s flavor period. After this period, a green 2 s cross cued the subjects to swallow. After this period, ratings were made with visual analog rating scales in which the subject moved a bar to the appropriate point on the continuous scale using a button box. Subjects rated the flavor stimuli on separate scales for pleasantness of flavor, for pleasantness of texture (with +2 being very pleasant and −2 very unpleasant), and for fattiness (with 0 being very low in fat and +4 being very high in fat). The subjects were instructed to rate the fattiness of the stimuli independently of how pleasant the stimuli were. Each rating period was 4 s long. Pre-experiment training in the protocol, and use of the rating scales allowed the participants to rate the pleasantness of texture separately from the fattiness of a stimulus. After the last rating, a small visual cue indicated the delivery of the tasteless control/rinse solution which was administered in exactly the same way as the test stimuli. Swallowing was again cued by a visual stimulus. The instruction given to the subject was to move the tongue once as soon as a stimulus or tasteless solution was delivered (at the time when a blue visual stimulus was turned on) to distribute the solution round the mouth to activate the receptors for taste, smell, and oral texture, and then to keep still for the remainder of the 7 s until a green cue indicated when the subject could swallow. There was then a 4 s delay period before the next trial started. Each experimental stimulus was presented in permuted sequence 12 times, interleaved with other trials on which thermal stimuli were applied to the hand as part of another investigation. For the fMRI analyses, the onset time for modeling the tasting period was delayed by 1 scan (2 s). The general protocol, design, and analysis methods have been used successfully in previous studies to investigate activations and their relation to subjective ratings in cortical areas [de Araujo et al., 2003a,b,c; Grabenhorst et al., 2008]. We note that alternative fMRI protocols for taste and flavor stimuli exist which focus on neural activity during swallowing periods [Buettner et al., 2001;

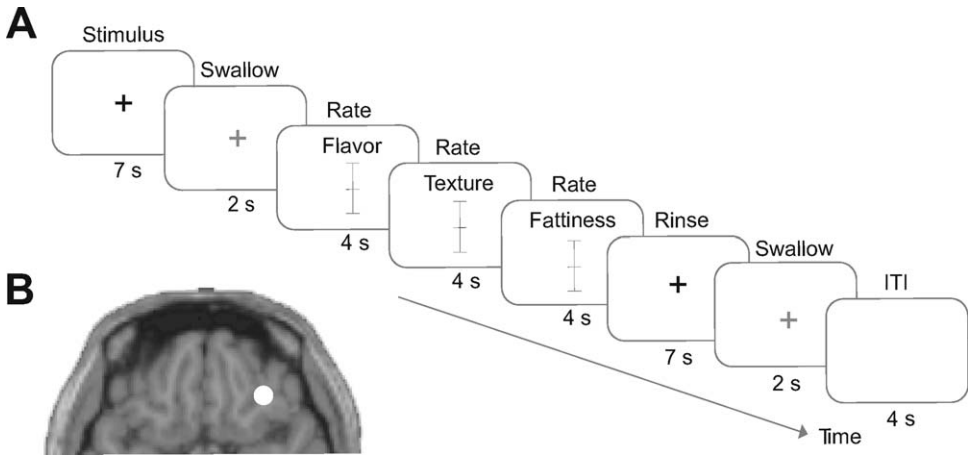

Figure 1.
Task design and seed area for PPI analyses. (**A**) Schematic of the trial structure. ITI: inter-trial interval. (**B**) OFC area [34 38 −10], where activity correlated with subjective pleasantness of oral fat texture and was stronger for high fat versus low fat vanilla-flavored stimuli [Grabenhorst et al., 2010]. This area was taken as seed region for PPI analyses.

Marciani et al., 2006]. However, for this investigation, we used those methods that are most commonly used in the field by different investigators [Bender et al., 2009; de Araujo and Rolls, 2004; de Araujo et al., 2003a; Grabenhorst and Rolls, 2008; Grabenhorst et al., 2008; McCabe and Rolls, 2007; Small et al., 2003, 2004; Veldhuizen et al., 2007].

Participants

Fourteen healthy volunteers (nine male and five female, mean age 24) participated in the study. Ethical approval (Central Oxford Research Ethics Committee) and written informed consent from all subjects were obtained before the experiment. The participants were asked not to eat for 3 h before the experiment, and the experiments were performed at approximately lunch time, so that the participants were sufficiently hungry to want to eat.

fMRI Data Acquisition

Images were acquired with a 3.0-T VARIAN/SIEMENS whole-body scanner at the Oxford Centre for Functional MRI of the Brain (FMRIB), where 27 T_2^* weighted echo planar imaging (EPI) coronal slices with in-plane resolution of 3×3 mm and between plane spacing of 4 mm were acquired every 2 s (repetition time (TR) = 2). We used the techniques that we have developed over a number of years [de Araujo et al., 2003a; O'Doherty et al., 2001] and as described in detail by Wilson et al. [2002] we carefully selected the imaging parameters to minimize susceptibility and distortion artifact in the OFC. The relevant factors include imaging in the coronal plane, minimizing voxel size in the plane of the imaging, as high a gradient switching frequency as possible (960 Hz), a short echo time of 28 ms, and local shimming for the inferior frontal area. The matrix size was 64×64 and the field of view was 192×192 mm. Continuous coverage was obtained from +62 (A/P) to −46 (A/P).

fMRI Data Analysis

Imaging data were analyzed using SPM5 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London). Preprocessing of the data used SPM5 realignment and unwarping, reslicing with sinc interpolation, normalization to the MNI coordinate system (Montreal Neurological Institute) [Collins et al., 1994], and spatial smoothing with a 6 mm full width at half maximum isotropic Gaussian kernel. The time series at each voxel were low-pass filtered with a hemodynamic response kernel. Time series non-sphericity at each voxel was estimated and corrected for [Friston et al., 2002], and a high-pass filter with a cut-off period of 128 s was applied. A general linear model (GLM) was then applied to the time course of activation where the onset of each oral stimulus was modeled with an impulse response function and convolved with the canonical hemodynamic response function [Friston et al., 1994]. Time derivatives were included in the basis functions set. The GLM included separate indicator functions for the following events: vanilla high fat stimulus, vanilla low fat stimulus, strawberry high fat stimulus, strawberry low fat stimulus, and for each of these trial types corresponding regressors modeling the swallowing periods, the three rating periods, and rinse periods. Parameter estimates for these regressors

were uniquely specified. For each of the four oral stimulus types, we included parametric regressors for the ratings of flavor pleasantness, texture pleasantness, and fattiness, to test for correlations between these ratings and neural activity. These three rating regressors were included in the same GLM without serial orthogonalization of the regressors, thereby effectively removing any shared variance components [Andrade et al., 1999; Draper and Smith, 1998]. In the second (group random effects) stage, subject-specific linear contrasts of parameter estimates were entered into one-sample *t*-tests. Comparisons involving the rinse control were defined between the experimental stimuli and the corresponding rinse control delivered within the same trial type ensuring orthogonality between rinse comparisons. This basic GLM was used to compare activations between flavor and rinse stimuli, and to test for correlations between neural activity and subjective ratings.

PPI models [Friston et al., 1997; Gitelman et al., 2003] tested how activity in pairs of brain regions was modulated by stimulus type. These included the following regressors: (1) the psychological regressor (vanilla high fat–vanilla low fat), (2) the psychological regressor (strawberry high fat–strawberry low fat), (3) a physiological regressor for the time series from the OFC seed region, (4) a PPI regressor between (1) and (3), and (5) a PPI regressor between (2) and (3). A second PPI model was identical to the first one except that the psychological regressors were (1) (vanilla high fat–strawberry high fat), and (2) (vanilla low fat–strawberry low fat). Time series were extracted from individuals' peak voxels within a 6 mm sphere around the group peak. The seed area used for the PPI analysis was a region of OFC in which we previously found a correlation with fat texture pleasantness ratings [Grabenhorst et al., 2010]. The specific area was located at [34 38 −10] where activity correlated with subjective pleasantness of oral fat texture and was stronger for high fat versus low fat vanilla-flavored stimuli [Grabenhorst et al., 2010]. Time courses were deconvolved following standard PPI procedures as implemented in SPM5 [Friston et al., 1997; Gitelman et al., 2003]. Contrast maps for the fourth and fifth (i.e., the PPI) regressor were entered into second-level analyses. A significant effect in a PPI analysis cannot reveal the direction of influences between pairs of brain areas, but can be interpreted as a modulation by the psychological effect (e.g., the high vs. low fat contrast) of the pattern of connectivity between two brain areas or as an effect of the activity in the seed brain area on the activation in the target area with respect to the psychological contrast [Friston et al., 1997; Gitelman et al., 2003].

We report effects that survive whole-brain correction ($P < 0.05$, family-wise error, cluster level) or, in brain regions with prior hypotheses, small volume corrections ($P < 0.05$, family-wise error). The corrected cluster size (spatial extent) threshold for whole-brain corrections was determined using the function corrClusTh.m provided by Thomas Nichols (http://www.sph.umich.edu/∼nichols/JohnsGems5.html). The cluster threshold sizes determined this way ranged from 159 to 335 voxels, depending on the specific statistical map. These brain regions included the anterior and mid-insula and somatosensory cortex (SSC) for which we corrected within spheres (10 mm radius) centered on coordinates from previous studies [de Araujo and Rolls, 2004; Eldeghaidy et al., 2011a; Miyamoto et al., 2006; Pardo et al., 1997; Veldhuizen et al., 2011a; Wang et al., 2002].

In addition to the whole-brain SPM regression analyses described above, which used subjective ratings as parametric modulators, we also tested for relationships between SSC activity and subjective ratings in supplementary region of interest (ROI) analyses as follows. First, for each subject, we extracted the blood oxygen level-dependent (BOLD) signal from the oral SSC using a 10 mm sphere centered on the average peak coordinates for oral SSC from prior studies. This ensured that the ROI coordinates were defined independently from other analyses. We then regressed the BOLD signal on the trial-by-trial ratings of flavor pleasantness, texture pleasantness, fattiness, and their interactions. This general procedure followed similar approaches used in previous fMRI studies [Behrens et al., 2008; Daw et al., 2006].

RESULTS

Psychophysical Data

As described previously [Grabenhorst et al., 2010], the high fat stimuli were rated as more pleasant in texture and more fatty than the low fat stimuli, and the vanilla stimuli were rated as more pleasant in flavor than the strawberry stimuli (main effects, within-subjects ANOVAs, all $P < 0.001$). The respective ratings for vanilla high fat, vanilla low fat, strawberry high fat, and strawberry low fat were for flavor pleasantness 1.38 ± 0.1, 1.20 ± 0.1, -1.34 ± 0.1, -1.31 ± 0.12; for texture pleasantness 0.98 ± 0.12, 0.45 ± 0.12, 0.07 ± 0.19, -0.34 ± 0.19; and for fattiness 2.83 ± 0.15, 1.63 ± 0.2, 2.2 ± 0.2, 1.3 ± 0.18. Because the aim of this investigation was to study neural processes related to oral fat texture when it is part of a pleasant flavor, the main fMRI analyses were performed separately for the vanilla and strawberry flavors. Within each flavor, the high fat stimulus was rated as more pleasant in texture and as more fatty than the low fat stimulus (vanilla: both $P < 0.001$, strawberry: both $P \leq 0.01$). Across both flavors, flavor pleasantness ratings did not differ between the high and low fat stimuli (nonsignificant main effect in an ANOVA). There was a significant interaction effect between flavor pleasantness and texture pleasantness ($P = 0.018$, ANOVA interaction effect) reflecting the fact that the combination of vanilla flavor and high fat content produced an especially pleasant rated texture (cf. Drewnowski [1995]). However, the effect size for this interaction effect was very small (partial-$\eta^2 = 0.002$) compared to the flavor main effect (partial-$\eta^2 = 0.91$). To control for differences in flavor pleasantness, we included the flavor

pleasantness ratings as a covariate in all relevant fMRI analyses.

Correlations between texture pleasantness and flavor pleasantness ratings within subjects and across trials were low and nonsignificant (average Pearson correlation of $r = 0.11$, with similar results if calculated separately for vanilla and strawberry flavors), indicating that subjects were able to independently rate the pleasantness of these different sensory properties of the stimuli. To further account for any collinearities between ratings, the GLM regression analyses on fMRI data were performed by including all three ratings (texture pleasantness, flavor pleasantness, and fattiness) as regressors in the same GLM without orthogonalization, thereby effectively removing shared variance components [Andrade et al., 1999; Draper and Smith, 1998].

fMRI Data

Previously, we showed that a part of the OFC was more strongly activated by high fat compared to low fat stimuli with pleasant vanilla flavor, and that activity correlated with texture pleasantness ratings [Grabenhorst et al., 2010] suggesting a representation of the reward value of oral fat texture. Here, we searched for brain areas that functionally interact with this OFC region in the hedonic analysis of fat texture. Therefore, we used PPI analysis to test for differential functional connectivity with the OFC seed area (Fig. 1B) during the consumption of pleasant-flavored high fat versus low fat stimuli. The data set used in this study was the same as used in the previous investigation [Grabenhorst et al., 2010]. The PPI analyses presented here are new and have not previously been reported.

Functional coupling between OFC and oral SSC

A whole-brain PPI analysis revealed that the BOLD signal in a region of the postcentral gyrus was more strongly correlated with signal in the OFC seed region for the pleasant high fat vanilla stimulus compared with the pleasant low fat vanilla stimulus (Fig. 2A; [66 −18 12], $t = 3.99$, $P = 0.039$, whole brain-corrected). The location of this effect was consistent with the oral part of SSC as it matched averaged coordinates from previous studies ([58 −14 30], see Methods).

The anatomical location of the oral part of SCC was confirmed in a control analysis which contrasted activations produced by the high and low fat flavor stimuli with activations produced by a tasteless rinse solution, following criteria used in previous studies [de Araujo et al., 2003a,c; Grabenhorst et al., 2008; Veldhuizen et al., 2007]. This analysis, contrasting all flavor stimuli with their respective rinse stimuli, showed bilateral effects in the same part of the postcentral gyrus with peak coordinates at [58 −22 20] and [−62 −10 22] ($t = 4.71$ and $t = 5.10$, respectively, both $P < 0.001$, whole brain-corrected, Fig. 2B). Consistent with previous findings [Grabenhorst et al., 2008; Small, 2008; Small and Prescott, 2005; Verhagen and Engelen, 2006], the comparison between flavor and rinse stimuli also showed significant effects in the anterior insular cortex, that is, the putative primary taste cortex ([30 18 8], $t = 4.92$, $P = 0.005$, whole brain-corrected). By contrast, significant PPI effects were not found in other areas including the anterior and mid-insular cortex.

Specificity of OFC-SSC coupling for pleasant-flavored high fat stimuli

Oral fat is particularly pleasant when combined with sweet taste and consonant flavors [Drewnowski, 1995]. Therefore, we tested whether OFC-SSC coupling was specific for fat texture when combined with pleasant flavor. The PPI term contrasting the less pleasant strawberry-flavored high fat versus low fat stimuli did not show a significant effect in oral SSC, even at a low threshold of $P < 0.01$, uncorrected. This indicated that increased OFC-SSC functional coupling was specific to high fat combined with pleasant flavor, and that high fat combined with less pleasant flavor did not modulate OFC-SSC connectivity. We performed a direct comparison of these effects to test statistically the specificity of OFC-SSC coupling for pleasant-flavored high fat stimuli. We found a significantly stronger PPI effect for the vanilla high versus low fat stimuli compared to the corresponding PPI effect for the strawberry high versus low fat stimuli in the SSC region (Fig. 2C; [64 −10 26], $t = 4.39$, $P < 0.05$, whole brain-corrected).

We also tested whether OFC-SSC coupling was modulated by simple differences in flavor pleasantness between low fat stimuli. The PPI term for the vanilla low fat compared to the strawberry low fat stimuli did not show a significant effect in SSC, even at a low threshold of $P < 0.01$, uncorrected. By contrast, the PPI term for the vanilla high fat stimulus compared to the strawberry high fat stimulus revealed a significant effect in oral SSC ([66 −20 12], $t = 3.85$ $P = 0.031$, small volume corrected). Indeed, in a direct statistical comparison of these effects we found a significantly stronger PPI effect for the vanilla high fat versus strawberry high fat stimuli compared to the PPI effect for the vanilla low fat versus strawberry low fat in oral SSC ([66 −14 25], $t = 4.77$ $P = 0.022$, small volume corrected).

Thus, enhanced OFC-SSC coupling was specific to stimuli that were a combination of high fat with pleasant sweet flavor, and did not occur for less pleasant high fat stimuli, or for pleasant-flavored low fat stimuli.

Relationship of oral SSC activity to subjective ratings

To characterize the representation of oral fat in the SSC, we regressed neural activity produced by the flavor stimuli on ratings of fattiness, texture pleasantness, and flavor pleasantness. Using the flavor-rinse contrast as an inclusive mask ($P = 0.005$, uncorrected), we found an indication

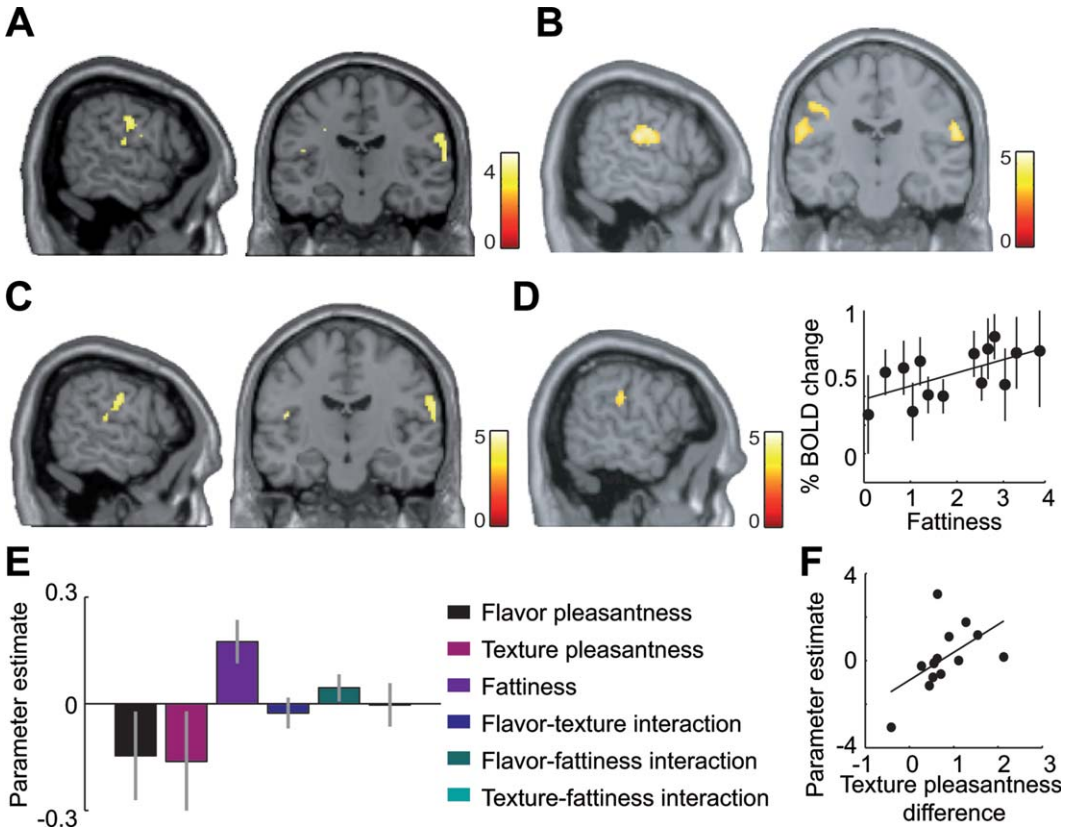

Figure 2.

Functional connectivity between oral SSC and OFC. (**A**) Stronger functional coupling between SSC and the OFC texture pleasantness area during consumption of pleasant high fat vanilla-flavored drinks compared to low fat drinks with the same flavor ([66 −18 12], $t = 3.99$, $P = 0.039$, whole brain-corrected). Color bar denotes *t*-scores. (**B**) Stronger SSC activation by flavor stimuli compared to a tasteless rinse solution ([58 −22 30], $t = 4.71$, [−62 −10 22], $t = 5.10$, whole-brain corrected [$P < 0.05$]). (**C**) Interaction effect: OFC-SSC coupling for pleasant, vanilla-flavored high fat versus low fat stimuli was significantly stronger than for less pleasant, strawberry-flavored high fat versus low fat stimuli ([64 −10 26], $t = 4.39$, $P < 0.05$, whole brain-corrected). (**D**) SSC activity correlated with fattiness ratings ([−58 24 26], $t = 3.85$, $P < 0.001$ uncorrected, and [66 −24 28], $t = 3.02$, $P < 0.005$, uncorrected, cluster threshold of five contiguous voxels). Right: The effect was confirmed in a ROI analysis on coordinates derived from previous studies. The scatter plot shows the relationship between the BOLD signal (% change) in SSC and subjective fattiness ratings across subjects ($r^2 = 0.37$, $P = 0.026$). (**E**) Parameter estimates from a multiple regression analysis of the BOLD signal in SSC on ratings of flavor pleasantness, texture pleasantness, fattiness, and their interactions. Only fattiness ratings had a significant relationship with the SSC BOLD signal ($P = 0.0036$). (**F**) OFC-SSC coupling across subjects (PPI parameter estimates, arbitrary units) correlated with differences in rated texture pleasantness between high and low fat stimuli ($r = 0.29$, $P < 0.004$, robust regression). Parameter estimates were extracted from a 10 mm sphere centered on coordinates from prior studies, ensuring independence from whole brain analyses.

that SSC activity bilaterally correlated with fattiness ratings (Fig. 2D; [−58 −24 26], $t = 3.85$, $P < 0.001$ uncorrected, and [66 −24 28], $t = 3.02$, $P < 0.005$, uncorrected, cluster threshold of five contiguous voxels). All ratings were included as covariates in the same GLM without regressor orthogonalization. This ensured that correlations with fattiness could not be explained by flavor or texture pleasantness. While this may have lowered statistical power, we note that the statistical threshold used followed criteria from previous fMRI studies [Gottfried et al., 2003]. Further, the fact that effects were found bilaterally considerably strengthened this finding.

To further explore and substantiate this finding, we performed ROI analyses. First, for each subject, we extracted the trial-by-trial BOLD signal from the oral SSC using a 10 mm sphere centered on the average peak coordinates for oral SSC from prior studies. This ensured that the ROI coordinates were defined independently from subsequent analyses. We then regressed the BOLD signal on the trial-by-trial ratings of flavor pleasantness, texture pleasantness,

fattiness, and their interactions. Multiple regression with the SSC timeseries as the dependent variable and all ratings and their interactions as independent variables showed a positive fattiness regressor ($P = 0.0036$), but no other significant effects ($P > 0.18$). Corresponding single regressions also showed a significant regressor ($r^2 = 0.37$, $P = 0.026$) with the ratings of fattiness, but no other effects (all $P > 0.11$). A scatter plot from this ROI analysis illustrating the relationship between the SSC BOLD signal and fattiness ratings across subjects is shown in Figure 2D. The parameter estimates (multiple regression betas) from the ROI analysis are show in Figure 2E. Thus, pleasantness ratings did not significantly explain variance in the SSC signal. Accordingly, SSC activity seemed related to sensory fattiness rather than hedonic stimulus properties (though future studies should test other measures of stimulus pleasantness, e.g., using satiety manipulations).

Relationship of OFC-SSC coupling to inter-individual differences in pleasantness ratings

Given that inter-individual differences in liking for high fat foods may be important in obesity [Bartoshuk et al., 2006; Drewnowski, 1998], we tested whether variation in OFC-SSC functional coupling across individuals explained variation in hedonic evaluations for high fat foods. Across subjects, the magnitude of the PPI effect in oral SSC for vanilla high fat versus low fat stimuli correlated with differences in texture pleasantness ratings between these stimuli (Fig. 2F; $r = 0.29$, $P < 0.004$, robust regression). Similar effects were not found for fattiness or flavor pleasantness ratings. We confirmed the robustness of this across-subjects analysis with a robust regression algorithm which used iteratively reweighted least squares [Holland and Welsch, 1977]. Further, the result remained significant ($P < 0.05$) even if outliers (data exceeding two standard deviations from the group mean) were removed.

DISCUSSION

Our findings indicate a specific role for the human SSC in the processing of pleasant-flavored oral fat. Functional connectivity analyses seeded from the OFC region, where the pleasantness of fat texture was represented, implicated the SSC in the representation of oral fat. Specifically, OFC-SSC coupling was stronger during consumption of high fat foods with a pleasant (vanilla, sweet) flavor, compared to low fat foods with the same flavor (Fig. 2A). Similar effects were not found for high fat foods with a less pleasant (strawberry) flavor or for pleasant-flavored low fat foods. These data indicate that a combination of high fat with a pleasant, sweet flavor specifically enhanced the functional coupling between the oral SSC and a brain area that represented the reward value of oral fat texture, the OFC. This is consistent with previous findings that supralinear responses to combinations of taste and olfactory, or taste and visual, stimuli are important in reward structures in the OFC and cingulate cortex [McCabe and Rolls, 2007; Rolls and McCabe, 2007; Small et al., 2004], and are also found in multisensory integration in other brain systems [Calvert et al., 2004].

Oral SSC activity was not directly related to subjective fat texture pleasantness or flavor pleasantness. Thus, there was no indication of a hedonic representation per se. Instead, SSC activity correlated with subjective fattiness (Fig. 2D,E), suggesting a representation of the sensory properties of oral fat. Although PPI analyses do not reveal the directionality of the connectivity, this finding may place the SSC at a similar processing stage as the anterior insular (primary taste) cortex (see diagram of the anatomical connectivity provided by Rolls [2012b]), where the pleasantness at least of taste is not represented neuronally in the primate brain [Rolls, 2011b, 2012b], and where fMRI signals are typically related to sensory properties of oral stimuli rather than their pleasantness [Grabenhorst and Rolls, 2008; Rolls, 2011b, 2012b; Small et al., 2003]. Although the anterior insula did not show a significant PPI effect, it could still be important by providing inputs about oral fat to OFC and oral SSC. This would be suggested by evidence for direct projections from the insular taste cortex to OFC [Baylis et al., 1995], neuronal representations of oral fat texture in insular primary taste cortex [Kadohisa et al., 2005a; Verhagen et al., 2004] and OFC [Rolls et al., 1999; Verhagen et al., 2003], activations to oral fat in these structures in the human brain [de Araujo and Rolls, 2004; Eldeghaidy et al., 2011a; Grabenhorst et al., 2010], and recent evidence by Kaas and colleagues that the tongue representation in oral SSC area 3b may receive projections from the anterior insula [Cerkevich et al., 2011].

We previously reported that activity in the pregenual cingulate cortex reflected the pleasantness of oral fat texture and flavor [Grabenhorst et al., 2010]. In this investigation, exploratory PPI analyses seeded from the pregenual cingulate cortex revealed only weak effects in the oral SSC which did not meet our criteria for statistical significance and which we, therefore, do not report. Anatomically, the pregenual cingulate cortex in primates does not receive strong projections from the somatosensory cortical areas [Carmichael and Price, 1995; Morecraft and Tanji, 2009], in contrast to the direct input from SSC to the OFC [Carmichael and Price, 1995]. Based on the strong reciprocal connections between OFC and pregenual cingulate cortex [Carmichael and Price, 1996; Morecraft and Tanji, 2009], we previously proposed that information about rewards, including oral fat texture, may reach the pregenual cingulate pleasantness area via the OFC [Grabenhorst and Rolls, 2011; Grabenhorst et al., 2010; Rolls and Grabenhorst, 2008]. We note that as a tertiary cortical taste area, beyond the OFC, the pregenual cingulate cortex may be less directly involved in the detailed analysis of the sensory properties of foods.

Previous studies found that functional connectivity between OFC, insula, and prefrontal cortex was modulated by cognitive factors, including selective attention, taste

expectation and detection, and hedonic judgments [Bender et al., 2009; Grabenhorst and Rolls, 2008, 2010; Veldhuizen et al., 2007, 2011b]. However, the presently observed OFC-SSC functional coupling occurred specifically for the vanilla high fat stimulus even though subjects performed identical hedonic and sensory evaluations for all stimuli. Further, we aimed to match semantic congruity between fat-flavor combinations using two flavors that are commonly consumed with fat (e.g., strawberry/vanilla milkshakes, ice cream, or strawberries with cream).

Overall, our findings open up the analysis of the role of the human SSC in the representation of oral fat. The functional coupling between the OFC and oral SSC reported here occurred specifically during the consumption of foods that had both high fat content and a pleasant flavor. This is consistent with evidence that oral fat is particularly pleasant when combined with consonant flavors [Drewnowski, 1998], and that liking for such foods can predict weight gain [Salbe et al., 2004]. Previous human imaging studies reported activations in SSC by different types of food stimuli [Cerf-Ducastel et al., 2001; Eldeghaidy et al., 2011a; Haase et al., 2009; Veldhuizen et al., 2007] and suggested links to obesity [Stice et al., 2008; Wang et al., 2002]. Further, obese individuals showed higher resting state activity of oral SSC [Wang et al., 2002] and youths and adults at risk for obesity showed higher activation of SSC by food stimuli [Stice et al., 2008, 2011]. Based on anatomical connections, Kaas and colleagues suggested an involvement of SSC in both oral tactile and taste processing [Kaas, 2005, 2012; Kaas et al., 2006]. Small [2008] proposed that the oral SSC participates in the process of oral referral and the binding of multimodal inputs into a flavor percept (cf. Eldeghaidy et al., [2011b]). Our findings extend these views to the processing of oral fat texture. Stice et al. [2011] posited that altered SSC responses in individuals at risk for obesity could reflect enhanced preferences for oral fat. Consistent with this proposal, we found that although the representation of oral fat in SSC appears to be of its sensory properties, the oral SSC is implicated in the hedonic analysis of fat texture via its coupling to the OFC region, where fat pleasantness is represented. Further, we found that inter-individual variation in this coupling explained variation in hedonic preferences between individuals. The present findings indicate, therefore, that alterations in the functional coupling between oral SSC and reward structures may constitute a potential neural mechanism that could contribute to individual differences in liking for high fat foods, and, therefore, to overeating and obesity. It will be important in future studies to investigate in more detail how OFC-SSC coupling strength across subjects is related to inter-individual differences on other measures of food liking, including food preferences as revealed by choices, and direct ratings of the overall experienced pleasantness of stimuli.

The seed area used for the PPI analyses in this study was selected based on a separate analysis in which OFC activity correlated with subjective pleasantness of oral fat texture and was stronger for high fat versus low fat vanilla-flavored stimuli [Grabenhorst et al., 2010]. We acknowledge that the seed area was thus not derived from an independent study. We note that this specific part of OFC has been implicated in food reward processing in many previous studies (see Grabenhorst and Rolls [2011] for a summary figure showing the overlap of effects from multiple studies).

Our findings identify a candidate mechanism by which combinations of pleasant flavor and high fat may contribute to the reward value of foods, via functional coupling of the SSC to the OFC. Previous studies showed that responses of the OFC and related reward areas to food stimuli can be modulated by food labels [de Araujo et al., 2005; Grabenhorst et al., 2008; Ng et al., 2011]. It will be of interest in future studies to investigate how food labels may interact with the sensory properties of specific foods designed to reproduce the pleasant mouth feel of fat, and whether the palatability and acceptance of such foods can be improved by providing appropriate labels.

These investigations provide a very interesting advance in our understanding of how oral fat texture, and its pleasantness, are represented in different brain areas [de Araujo and Rolls, 2004; Grabenhorst and Rolls, 2011; Grabenhorst et al., 2010; Kadohisa et al., 2005a,b; Rolls, 2011a]. The findings are of potential importance in understanding the mechanisms of appetite control and their disorders including obesity [Rolls, 2011b, 2012b], and in the design of foods that produce the mouth feel of fat but have a low energy value [Rolls, 2011a, 2012a].

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