NeuroImage 113 (2015) 298–309

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Age differences in the brain mechanisms of good taste

Edmund T. Rolls [a,*], Michele B. Kellerhals [b], Thomas E. Nichols [c]

[a] Oxford Centre for Computational Neuroscience, Oxford, UK
[b] Research & Development, Coca-Cola Services, Brussels, Belgium
[c] Department of Statistics, University of Warwick, Coventry CV4 7AL, UK

ARTICLE INFO

Article history:
Received 9 September 2014
Accepted 25 March 2015
Available online 2 April 2015

Keywords:
Reward
fMRI
Taste
Flavor
Food
Orbitofrontal cortex
Cingulate cortex
Cognitive modulation

ABSTRACT

There is strong evidence demonstrating age-related differences in the acceptability of foods and beverages. To examine the neural foundations underlying these age-related differences in the acceptability of different flavors and foods, we performed an fMRI study to investigate brain and hedonic responses to orange juice, orange soda, and vegetable juice in three different age groups: Young (22), Middle (40) and Elderly (60 years). Orange juice and orange soda were found to be liked by all age groups, while vegetable juice was disliked by the Young, but liked by the Elderly. In the insular primary taste cortex, the activations to these stimuli were similar in the 3 age groups, indicating that the differences in liking for these stimuli between the 3 groups were not represented in this first stage of cortical taste processing. In the agranular insula (anterior to the insular primary taste cortex) where flavor is represented, the activations to the stimuli were similar in the Elderly, but in the Young the activations were larger to the vegetable juice than to the orange drinks; and the activations here were correlated with the unpleasantness of the stimuli. In the anterior midcingulate cortex, investigated as a site where the activations were correlated with the unpleasantness of the stimuli, there was again a greater activation to the vegetable than to the orange stimuli in the Young but not in the Elderly. In the amygdala (and orbitofrontal cortex), investigated as sites where the activations were correlated with the pleasantness of the stimuli, there was a smaller activation to the vegetable than to the orange stimuli in the Young but not in the Elderly. The Middle group was intermediate with respect to the separation of their activations to the stimuli in the brain areas that represent the pleasantness or unpleasantness of flavors. Thus age differences in the activations to different flavors can in some brain areas be related to, and probably cause, the differences in pleasantness of foods as they differ for people of different ages. This novel work provides a foundation for understanding the underlying neural bases for differences in food acceptability between age groups.

Introduction

There are age-related differences in the acceptability of different foods. For example children may not take readily to a wide range of vegetables, yet find sweet foods palatable (Birch, 1999; Hetherington et al., 2011). Adults may find a wide range of foods pleasant. As people age, smell and even taste may become less sensitive (for example when tested with stimuli close to threshold) (Jacobson et al., 2010; Murphy, 1993; Murphy et al., 2002; Stevens et al., 1995), and this may contribute to the changes in eating that can occur in aging (Green et al., 2011; Murphy, 1989; Rolls, 1999). However, age-related changes in the ability to discriminate foods at the sensory threshold – the point at which we can just detect tastes – may not be the main factor that accounts for why people's propensity to different foods is different at different ages, because most foods have ingredients that are far above the detection threshold. Therefore we did not attempt to measure effects close to threshold in the different age groups, but instead used the real foods and beverages described. In order to examine the neural mechanisms underlying the age-related differences in the acceptability of different flavors and foods, we performed the fMRI study described here with three different age groups (termed Young, mean age 22 years; Middle, mean age 40; and Elderly, mean age 60) and used foods, that is stimuli that include different taste, olfactory, and texture components, rather than pure tastes (such as sweet or bitter) which have been used in some previous studies (Green et al., 2013; Jacobson et al., 2010), because we are interested in responses to real, ecologically valid, foods. Indeed, in previous studies we have shown that the convergence of taste and odor to produce flavor occurs anterior to the insular taste cortex in the agranular insular cortex and areas that receive from it including the orbitofrontal cortex and anterior cingulate cortex (de Araujo et al., 2003c; Rolls, 2015c,e); and that the flavor formed by convergence of a taste such as monosodium glutamate and an odor such as vegetable odor can produce a flavor much more pleasant than the components, which is reflected in activations in the orbitofrontal and anterior cingulate cortex (McCabe and Rolls, 2007). Further, we were interested in how cognitive descriptions of foods, such as "no

* Corresponding author.
E-mail address: Edmund.Rolls@oxcns.org (E.T. Rolls).
URL: http://www.oxcns.org (E.T. Rolls).

http://dx.doi.org/10.1016/j.neuroimage.2015.03.065
1053-8119/

added calories", and "nutritionally relevant for the elderly" might influence the responses of different brain regions to foods in the different age groups, given that we have shown that there are strong effects of cognitive descriptions on the responses of some brain regions to odor (de Araujo et al., 2005), and to taste and flavor (Grabenhorst et al., 2008).

A further important aim of this investigation was to measure the extent to which responses of brain systems involved in the identification and intensity processing of a food differ from those involved in determining the pleasantness in people of different age groups, for it is likely to be the processing in pleasantness (hedonic or affective) systems that drives food intake (Rolls, 2012b, 2014a, 2015d,e). Indeed, in the primate including human brain, there are two partly independent systems for analyzing taste, olfaction, and flavor. One system is for the intensity and identity of taste (the insular primary taste cortex in the anterior dorsal granular insula and adjoining frontal operculum) and odor (pyriform primary olfactory cortex), where activations correlate with intensity and not pleasantness ratings, and where feeding to satiety does not reduce neural activity (Grabenhorst and Rolls, 2008; Rolls, 2012b, 2014a; Rolls et al., 1988, 2008; Yaxley et al., 1988). These areas project into the agranular insular cortex just anterior to the primary taste cortex to produce flavor representations (de Araujo et al., 2003c). The second main system (which receives input from the agranular insula, and the insular primary taste cortex and the pyriform primary olfactory cortex) is the orbitofrontal cortex, which then projects onto the anterior cingulate cortex. Activations in both the orbitofrontal cortex and anterior (pregenual) cingulate cortex are linearly correlated with the subjective pleasantness of the taste, odor, or flavor (Grabenhorst and Rolls, 2011; Rolls, 2005, 2012b; Rolls and Grabenhorst, 2008). These taste, olfactory, and flavor processing pathways are illustrated in diagrams available elsewhere (Grabenhorst and Rolls, 2011; Rolls, 2012b, 2014a, b, 2015c, e; Rolls and Grabenhorst, 2008).

Given these aims, the research questions and associated hypotheses to be tested included the following: H1. For sweet and savory whole food stimuli (that include taste, olfactory, and texture components), are there age-related differences in activations in the insular primary taste cortex that are related to the liking (measured by subjective pleasantness) of the different foods? H2. For sweet and savory whole food stimuli, are there age-related differences in activations in the agranular insula, orbitofrontal cortex, amygdala, and anterior cingulate cortex that are related to the liking (measured by subjective pleasantness) of the different foods? H3. How do differences in different age groups in brain processing that are related to pleasantness separate from any differences in the processing of the intensity of the stimuli? H4. Do cognitive descriptions of foods influence differently in different age groups the responses of different brain systems to foods? To test H4 we compared responses to the identical flavor (Fanta®) when it was labeled as the regular energy Fanta® and as the low energy orange flavor drink (Fanta® Zero) that was labeled "no added calories". It was hypothesized that this might have an effect especially in the Young group. We also tested H4 by comparing responses to identical vegetable juice, but in one case providing a cognitive description claiming that it contained ingredients useful for healthy aging. It was hypothesized that this might have different effects in the Elderly vs the Young group.

We had prior hypotheses about the locations in the brain where different types of flavor-related activation would be found, as follows (Grabenhorst and Rolls, 2011; Rolls, 2012b, 2014a, 2015c,e; Rolls and Grabenhorst, 2008). Activations to taste, flavor, and texture stimuli are found in the primary taste cortex in the anterior dorsal granular insula and adjoining frontal opercular cortex (de Araujo et al., 2003a; de Araujo and Rolls, 2004; Small, 2010), and these activations reflect the subjective intensity of the stimuli (Grabenhorst and Rolls, 2008) and can be influenced by top-down processing (Bender et al., 2009; Ge et al., 2012; Grabenhorst and Rolls, 2008; Luo et al., 2013; Nitschke et al., 2006). Activations to taste, odor, and their combination to produce flavor, are found in the agranular insular cortex (de Araujo et al., 2003c; Small and Prescott, 2005). Activations to the hedonic value of taste, flavor, and texture stimuli are found in the orbitofrontal cortex, with activations medially related to the pleasantness of the stimuli, and more laterally to the unpleasantness of the stimuli (Grabenhorst and Rolls, 2011). Activations to taste and flavor stimuli are found in the amygdala (which receives inputs from the primary taste and olfactory cortices (Rolls, 2014a)), with activations that are produced by pleasant and by unpleasant stimuli (Kadohisa et al., 2005a; Karadi et al., 1998; O'Doherty et al., 2001). Activations to the hedonic value of taste, flavor, and texture stimuli are found in the anterior cingulate cortex, with activations in the pregenual cingulate cortex related to the pleasantness of the stimuli, and in the anterodorsal cingulate cortex to the unpleasantness of the stimuli (Grabenhorst and Rolls, 2011; Rolls, 2015a).

We note that as commonly used, "taste" applied to foods includes at least gustatory components as defined by taste receptors sensitive to sweet, salt, bitter, sour, and umami; olfactory components sensed by the olfactory receptors in the nose; and texture components, including oral viscosity, fat texture, and grittiness (Rolls, 2012b, 2015c,e). Together, these components, with further contributions of the sight of the food and cognitive effects such as the verbal description of the food, produce what is often termed flavor. The stimuli used in this investigation were real foods and beverages, so included these components, and it is the neural bases of these flavor effects that are being investigated. For simplicity and consistent with natural language usage, we sometimes refer to the stimuli as taste stimuli, but for the stimuli used in this investigation, note that flavor as defined above is a more technical term that applies to these stimuli.

Methods

Design of the investigation

Volunteers in three age groups were scanned using fMRI while different drinks were delivered with different picture and label descriptions to investigate whether the representations of the reward value of these beverages in the brain changes as a function of age.

The three age groups recruited were: 18–26 (Young, Y); 34–46 (Middle, M); and 53–67 (Elderly, E) years of age, with further description under Participants. One of the beverages used was the soft drink Fanta®, which is highly acceptable to the Young group, but consumed less by the older groups. It was provided in two forms which have the same flavor but different labels which may influence different groups differently, Fanta® and Fanta® Zero. The third Fanta stimulus was the flavor without a labeled picture, and was incorporated in case it helped to reveal cognitive effects. Brain activations to these were compared to orange juice (which is pleasant and is known to be consumed by most age groups so forms a useful reference stimulus). Comparisons were also made to a savory drink, a tomato flavor vegetable drink, V8®, because this is more likely to appeal to the Elderly group and is potentially suitable for incorporating health-promoting ingredients with labels designed to appeal to the Elderly group. The V8 was provided in two forms, one with a cognitive label emphasizing attributes that might be of especial interest to the elderly, and the other with the same picture of V8 but without the same cognitive label.

The design was to recruit for each group 20 participants, 10 males and 10 females, to allow between-group statistical comparisons. The subjects were recruited from Reading, UK. All stimuli were purchased in the UK, and were as follows, with the images displayed during the delivery of the beverages as described.

T1. Fanta® with a picture label of a can of Fanta® designed to ensure that we are measuring brain responses in this condition to a beverage identified as Fanta®.

T2. Fanta® with a picture label of a can of Fanta® zero and the words "No added sugar" designed to appeal to those concerned with their energy intake.

T3. Fanta® delivered with no picture label but an isoluminant screen. This was to measure the brain responses to the flavor per se, when the product was not known to be Fanta®.
T4. Orange juice (Tropicana®). This was chosen as a comparison or anchor stimulus, as a beverage in which the acceptability changes relatively little in people in different age groups. The picture label was an image of a glass of orange juice, and the words "100% freshly squeezed orange juice".
T5. V8® vegetable juice. A vegetable/health food beverage that was accompanied by a picture label of a can of V8® and words indicating that it contains nutritious health-enhancing ingredients. This stimulus was included to enable measurement of whether this type of beverage activates reward systems in the brain more with aging, because people are attracted to foods with nutritional benefits for their brain health. The V8® has a flavor of tomato juice. The label words included "Excellent source of vegetable nutrients such as potassium, magnesium and vitamins A, C and E".
T6. V8® vegetable juice. The same vegetable/health food beverage but accompanied only by a picture label of a can of V8®, to act as a comparison condition for T5 in order to investigate the cognitive effects of the health-related description in T5 on the responses of different brain regions in the different age groups.
It is emphasized that the beverage delivered on T1–3 was identical, orange flavored Fanta®; and on T5–6 was V8®. The differences between the conditions were in the pictures/labels that were shown when the beverage was delivered. This enabled effects of the labels on the brain's responses to the flavors to be measured.

Stimulus delivery

A rinse stimulus was delivered between each delivery of a flavor stimulus. This was done not only to rinse the mouth between flavors, but also to provide a reference control stimulus in which no taste or flavor was delivered, but the somatosensory effects and the mouth movement associated with the delivery of a flavor stimulus could be controlled for. 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 a flavor stimulus allowed flavor (taste, olfactory, and oral texture) effects to be distinguished from general somatosensory effects produced by introducing fluid into the mouth and any mouth including tongue movement (de Araujo et al., 2003a,b,c; O'Doherty et al., 2001).

Flavor stimuli were delivered at room temperature of 21C to the subject's mouth through four Teflon tubes (one for each of the 3 flavor stimuli, and one for the tasteless rinse control; the tubing was HW9 3 mm × 0.5 mm wall) that were led into the mouth by a single orifice to eliminate dead space. Each Teflon tube of approximately 6 m in length was connected to a separate computer driven motorized syringe pump located in the control room. (The pumps were Aladdin Model, World Precision Instruments, Inc., with a 60 ml Terumo syringe driven via a National Instruments NI-USB 6009 interface by computer programs written using NBS Inc Presentation software.) The fully automated flavor delivery system was set up to deliver 1.2 ml of any flavor stimulus or the rinse over a 1.9 s period at the time indicated by the computer.

Each trial started with a 7 s flavor period (see Fig. 1). For the first 1.9 s of this period, a 1.2 ml flavor stimulus was delivered into the mouth, and it remained in the mouth. For the whole 7 s period, one of the images defined under stimuli was shown. Then at $t = 7$ s (with $t = 0$ as the start of the trial) a different visual stimulus containing just the word "swallow" was shown for 2 s, and the participant swallowed the flavor. After this period, ratings were made with visual analogue 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 first for the pleasantness of flavor (with +2 being very pleasant and −2 very unpleasant), and then for the intensity of the flavor (with 0 being very weak and +4 being very intense). The subjects were instructed to rate the pleasantness of each stimulus when it was delivered on that trial, and to keep these ratings independent of the intensity of the stimulus on that trial. Each rating period was 5 s long. Pre-experiment training in the protocol, and use of the rating scales allowed the participants to rate the pleasantness of flavor separately from the intensity of a stimulus. (These scales and the protocol have been used extensively in previous investigations (de Araujo et al., 2003a; de Araujo et al., 2003b; de Araujo et al., 2003c; Grabenhorst et al., 2008; Grabenhorst et al., 2010b).) At $t = 19$ s a 7 s rinse period occurred, with an equiluminant visual stimulus containing the word 'rinse' indicating rinse delivery shown for the full period, and the delivery of the tasteless control/rinse solution administered in exactly the same way as the flavor stimuli with 1.2 ml in the first 1.9 s period. This volume was used based on previous investigations as being sufficient to produce good neural taste responses (de Araujo et al., 2003a, b,c; Grabenhorst et al., 2008, 2010b), as being easy to swallow in the test situation, and so that the total volume of fluid ingested did not influence hunger. At $t = 26$ s swallowing was again cued by a 2 s 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 visual stimulus was turned on) in order 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 the visual stimulus indicated when the subject could swallow. There was then a 2 s delay period before the next trial started. Each trial thus had a duration of 30 s, as shown in Fig. 1.

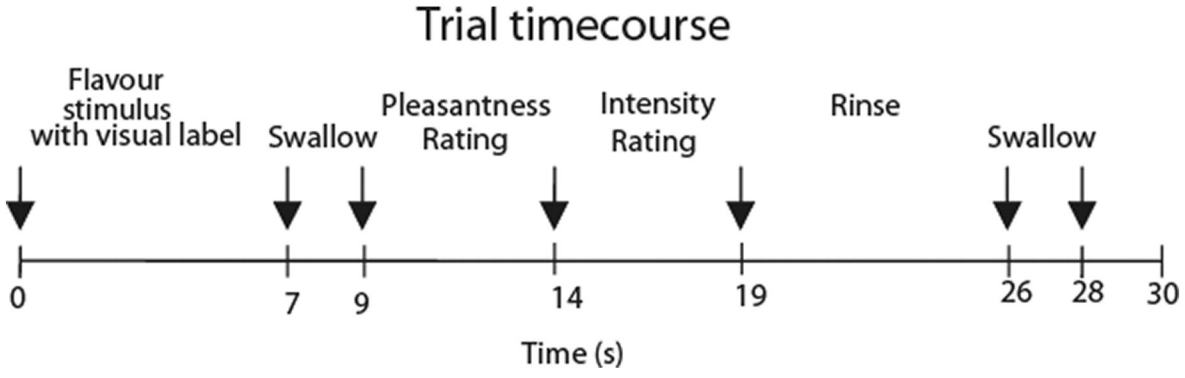

Fig. 1. The timecourse of each trial.

All participants were provided with practice with the stimuli and rating procedures inside the scanner before the scanning started, and the intensity and pleasantness ratings were checked for every subject for every stimulus before the scanning started to ensure that the stimuli were being rated correctly and reasonably for both pleasantness and intensity. (If for example despite the screening, the subject rated any stimulus as very unpleasant at −2, they were excluded from the investigation.)

Each experimental stimulus was presented in permuted sequence 12 times. This general protocol and design has 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, 2010b). A scanning session consisted of 12 presentations of the 6 stimuli, that is 72 trials, and each trial lasted 30 s.

Participants

66 healthy volunteers participated in the study. Results from the first 9 scanning sessions were not included in the data analysis, as these sessions were used to set up the protocols, including scanning parameters to allow all the regions of the brain of potential interest to be scanned including the insular taste cortex, orbitofrontal cortex, cingulate cortex, and amygdala. After allowance for these sessions, the results are based on 57 subjects who completed a scanning session with 3 groups: 18–26 years of age (Young, Y, 18 participants); 34–46 (Middle, M, 20 participants); and 53–67 (Elderly, E, 19 participants). (The means and standard deviations of the ages were Young 22.2 ± 3.1 years; Middle 39.8 ± 4.1; Elderly 59.7 ± 3.9). There were equal numbers (within 1) of males and females within each group, and the participants were mainly UK nationals recruited from Reading, UK. All participants had a body mass index of less than 30 (as obesity may influence taste (Green et al., 2011)), and smokers, and those with psychiatric or neurological disorders or diabetes were excluded from the study. In addition, all participants were pre-screened to ensure that all the stimuli were acceptable to them to consume while being scanned. Further, all subjects discriminated well between the stimuli, as shown by their ratings of the intensity and pleasantness of the different flavors made on every trial during the scanning.

Ethical approval (University of Reading Research Ethics Committee, Reading, UK) and written informed consent from all subjects were obtained before the experiment. The participants were asked not to eat or drink for 2 h before the experiment.

fMRI data acquisition

Images were acquired with a 3 T Siemens Trio scanner at the Centre for Integrative Neuroimaging and Neurodynamics, University of Reading, Reading, UK. 42 T2* weighted EPI slices with in-plane resolution of 2 × 2 mm and 2.5 mm thick (no skip) were acquired every 2.5 s (TR = 2.5). We optimized techniques for the Trio to obtain good signal to minimize signal dropout due to susceptibility and to minimize distortion artefact from the orbitofrontal cortex, cingulate cortex, and amygdala, using systematic exploration of parameters suggested by previous investigations (Deichmann et al., 2002, 2003; Weiskopf et al., 2006, 2007). After considerable exploration, we found the following to meet the requirements well: imaging plane −30 deg axial; Phase A >> P; TE = 25 ms; TR = 2500 ms; FOV 192 mm, 2 mm "pixel spacing" in 92 × 92 image (in plane pixel size 2 × 2 mm); 2.00 mm slice thickness, 0.5 mm skip (center-to-center slice spacing 2.5 mm); 42 slices (as many as was possible for the TR and TE); NORM prescan option; Echo Spacing 0.82 ms. Pixel Bandwidth 2264; Bandwidth per pixel phase encode 25.72; shimming: advanced. Structural scans were acquired using a T1-weighted MP-RAGE sequence with fat suppression in an axial orientation, 0.9765625 × 0.9765625 × 1 mm voxels, TR 2020 ms, TE 2.9 ms, TI 1100 ms, and FA 9 deg.

fMRI data processing

The overall design of the analyses was to use SPM8 analyses (described below) to identify three types of brain site across all participants: 1) sites where activations reflected a taste – rinse effect; 2) brain sites where the activations were positively correlated with the pleasantness ratings made on every trial; and 3) brain sites where the activations were negatively correlated with the pleasantness ratings (i.e. were positively correlated with unpleasantness). The second step was at those sites to extract the blood oxygenation-level dependent (BOLD) response change produced by the six stimulus conditions in each subject, and then to perform ANOVAs (using SPSS) on the BOLD signals to investigate how each site responded to the six stimuli in each of the three groups.

The imaging data were first pre-processed using FSL (FMRIB's Software Library, version 4.1.9) for spatial preprocessing, and using SPM8 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London) for statistical modeling. (It was found that FSL's epi_reg tool was needed to reliably coregister the oblique-axial functional data to each subject's structural T1 image.) Pre-processing of the data consisted of motion correction, functional–structural intrasubject registration, and intersubject registration (Smith et al., 2004) to the MNI coordinate system (Montreal Neurological Institute) (Collins et al., 1994), and spatial smoothing with a 8 mm full width at half maximum isotropic Gaussian kernel given the expected scale of the extent of activation.

Then using SPM8, "first level" (intra-subject) time series models were fit. These models accounted for low frequency drift with a Discrete Cosine Transform basis (128 s cut-off), and temporal autocorrelation with an approximate, global auto-regressive order-1 model (Friston et al., 2002); a set of 24 regressors based on the motion correction parameters were included to reduce motion-related variance (Lund et al., 2006). For each subject three "first level" statistical models were fit, a tastant-specific model (where tastant refers here to the flavor stimuli T1–6), a model involving correlations of the activations with the subjective pleasantness ratings made on every trial, and a model involving correlations of the activations with the subjective intensity ratings made on every trial. The tastant-specific model modeled the response of each of the 6 tastant types, where the onset of the oral stimulus effects (at $t = 2.5$ s in each trial) was modeled as an instantaneous event (0 s duration in SPM) and then convolved with the canonical hemodynamic response function (Friston et al., 1994); time derivative regressors were also included for each condition type to allow variation in the event onset. (The oral stimulus was delivered at $t = 0$ s, and the 2.5 s delay for the analysis was to allow the oral stimulus to be delivered by the pumps in their 1.9 s pumping time, for the liquid to be distributed in the mouth by the single tongue movement, and for the taste effects to become apparent in the fMRI signal.) For the correlational models, all tastants were modeled with a common response as well as a "parametric modulation" response regressor whose intensity was scaled by a behavioral response for that trial; specifically, one model fit the pleasantness-related variation in tastant response, and another the intensity-related variation. Linear contrasts were defined to test specific effects, producing "contrast" maps, the first-level effect estimates suitable for group modeling. The effects of taste – rinse were assessed at the first (individual subject) level, and this contrast was entered into the second level (group) analyses.

The primary analyses of interest are ROI-based inferences on tastant or age group differences. We selected regions for subsequent analysis using one-sample *t*-tests pooling over tastants and groups. When group sizes are equal, across-group averages are statistically independent of group differences, thus alleviating concerns about circularity on subsequent analyses using these locations (Kriegeskorte et al., 2009). Here, our group sizes are nearly but not exactly balanced (n's of 18, 20, 19) and thus we conducted numerical simulations to confirm that the group difference false positive risk was not inflated when

using the main effect as a filter.[1] For the Taste–Rinse contrasts (Figs. 4 and 5), these whole-group results are reported at whole brain FWE-corrected $p < 0.05$ (family wise error, FWE), while correlations with subjective ratings (Figs. 6 and 7) are shown at small volume correction (svc) FWE-corrected $p < 0.05$ for a sphere of 8 mm based on prior hypotheses. (We confirmed that the svc results reported were also significant $p < 0.05$ FWE when the svc was performed on the cluster, reducing reliance on exact coordinates from previous studies. Also, note that the less stringent significance criterion for defining regions for subjective ratings correlations does not invalidate the subsequent group comparisons.) The brain regions with prior hypotheses were: regions within the orbitofrontal and anterior cingulate cortex, anterior and mid-insular cortex, ventral striatum, hypothalamus, and amygdala in which we and others have found activations in previous studies to taste and flavor stimuli (de Araujo et al., 2003a,b; de Araujo and Rolls, 2004; Grabenhorst et al., 2008, 2010a,b; Grabenhorst and Rolls, 2008, 2010; Iannilli et al., 2012, 2014a,b; Kringelbach and Rolls, 2004; O'Doherty et al., 2001; Rolls, 2005, 2006; Small et al., 1999, 2003; Small and Prescott, 2005; Veldhuizen et al., 2011), and as receiving taste or olfactory inputs based on anatomical and neurophysiological evidence (Rolls, 2008a, 2012b, 2014a). In additional exploratory analyses, a 1-way ANOVA model for the young, middle aged and elderly groups was used to identify significant age-related modulation in fMRI BOLD effects.

The subsequent analysis was focused on brain regions identified as described above. At the identified coordinates, the mean fMRI BOLD signal for each flavor condition was extracted in a 6 mm radius sphere consisting of 143 $2 \times 2 \times 2$ mm voxels and exported to SPSS for analysis. (Due to the 8 mm spatial smoothing the effective kernel size was slightly larger.)

The activations shown in the figures are the BOLD signal change to the Tastant minus that to the Rinse. The activations were the BOLD signal change measured by the parameter estimates (betas) in SPM8, and are thus on an arbitrary scale (see http://blogs.warwick.ac.uk/nichols/entry/spm_plot_units/ written by one of the authors, T.E.Nichols; using scalefactors from the grand mean image, model and contrasts, in this study the plotted parameter estimates can be converted to approximate percent BOLD change by multiplication by 0.08). The use of a tasteless rinse allows measurement of brain responses to a taste when any effect of the mouth including tongue movement and the somatosensory effect produced by the delivery of the taste into the mouth has been subtracted (de Araujo et al., 2003a,b,c; O'Doherty et al., 2001). Of course, if what is delivered into the mouth has a texture, such as the V8 vegetable juice, or the carbonation of the Fanta, then this will not be subtracted out, as the tasteless solution has the viscosity and texture of water.

Statistical analysis of ROI-extracted data

Brain sites were identified in SPM8 for further analysis of the BOLD signal changes to the different stimuli based on the following procedures performed in SPSS. For each ROI, these statistical tests used repeated measures ANOVA to compare tastants within each age group (one intra-subject factor of stimulant; in SPSS, a "one-way within-subjects ANOVA"), as well as with tastant as one factor and age group as the second factor (one inter-subject factor of group, one intra-subject factor of stimulant; in SPSS, a "two-way mixed factorial ANOVA"). The results of these analyses are illustrated in Figs. 4–7 by the group means activations in each age group to each of the 6 stimuli. The error estimate shown in these figures is only to provide a rough visual indicator of variability within each age group, and is computed as ± half the standard error of the mean and avoids over-plotting issues.

[1] Monte Carlo simulation details. 100,000 3-group datasets of sizes 18, 20, & 19 were simulated, as standard normal variates. To reflect the selection that occurs with filtering with the main effect, we only considered the subset of realizations that had a significant one-sample *t*-test on the whole 57-subject dataset at $\alpha = 0.05$. For this subset, we measured the false positive rate (nominal $\alpha = 0.05$) for tests of pairwise differences between the group. The worst false positive rate was 5.1%, within the 95% Monte Carlo confidence interval based on the number of the selected tests. This confirms that there are no problems with circularity, as would be the case for a perfectly balanced sample.

Results

The activations shown in the figures below are the change in the signal measured with fMRI, the BOLD (blood oxygenation-level dependent) signal. All the BOLD responses shown have the response to the tasteless rinse control subtracted (see Methods).

Subjective pleasantness and intensity ratings

The subjective pleasantness and intensity ratings of the different stimuli in the 3 age groups are shown in Figs. 2 and 3. These are the subjective ratings made during the brain scanning just after each stimulus was being delivered, and were used to identify for example brain regions where the pleasantness of the stimuli were represented using regression analyses of the change in the BOLD signal against the subjective ratings. The main effects evident in the subjective pleasantness ratings were that the different age groups rated Fanta® as being similarly pleasant, and of comparable pleasantness to the orange juice; and that the V8® vegetable juice was rated as pleasant by the elderly group, less pleasant by the Middle Group, and unpleasant by the Young group. This was supported by the statistical analyses. A two-way repeated measures ANOVA on the pleasantness ratings across all 6 stimuli showed that there was a significant age × taste interaction ($F_{[10,270]} = 4.45$, $p << 0.001$). An ANOVA on the pleasantness ratings across the four sweet stimuli Fanta T1–T3 and Orange T4 showed that there was no significant age × taste interaction ($F_{[6,162]} = 1.67$, $p = 0.09$).

There was a small effect for Fanta with the picture of standard Fanta® (T1) to be rated as more pleasant than Fanta with the picture of Fanta Zero® and the label 'no added sugar' (T2). This was significant in a within subjects ANOVA across these 3 stimuli ($F_{[2,108]} = 7.28$, $p = 0.001$; with no age × taste interaction). This was of interest, for the Fanta stimulus samples delivered into the mouth were identical for T1–T3, and it was only the accompanying image that was different.

There were no significant effects of age on the intensity ratings to the different stimuli (Fig. 3) ($F_{[10,270]} = 1.67$, $p = 0.09$). The standard Fanta (T1) where an image of a standard can of Fanta was shown was rated as a little more intense than the Fanta with an image of a can of Fanta zero and the words "no added sugar" (T2), and than the Fanta without any

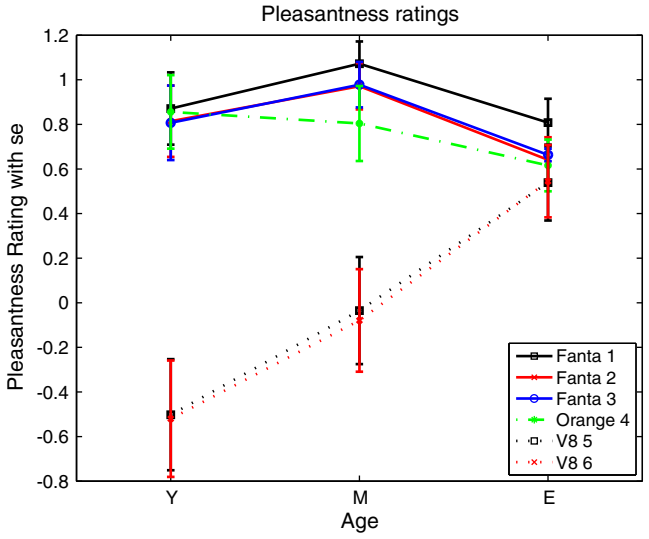

Fig. 2. Pleasantness ratings made during the scanning with 12 trials for each stimulus, mean ± standard error. Very pleasant = +2, neutral = 0, very unpleasant = −2. Age groups: Y = Young, M = Middle, E = Elderly.

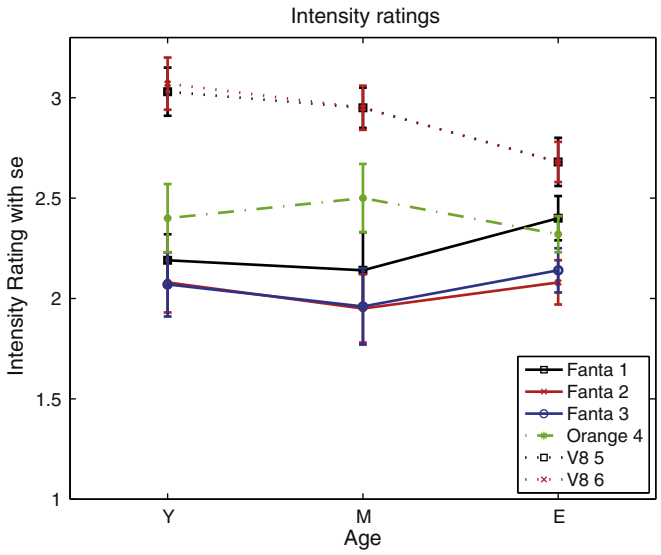

Fig. 3. The intensity ratings made during the scanning with 12 trials for each stimulus, mean ± standard error. Very intense = 4, midrange = 2, very weak = 0. Age groups: Y = Young, M = Middle, E = Elderly.

image or label (T3), even though the liquids delivered were identical ($F_{[2,108]} = 18.27$, $p << 0.001$).

Activations in brain regions identified by a response to the flavor minus the rinse

These sites were identified by significant activations in the SPM analysis to the contrast flavor minus rinse (with whole brain FWE $p < 0.001$ unless otherwise stated in Table S1). Flavor here indicates all the stimuli, T1–T6, so that texture effects from the V8 were included. The effects of the tasteless rinse delivered on every trial during the functional neuroimaging were subtracted from the effects of the taste so that non-taste-related effects such as the single mouth movement made to distribute the taste in the mouth after it was delivered were not reflected in the results.

Insular taste cortex

The activations are illustrated in Fig. 4, and were found at [−36 16 2]. This is a region that has been identified as the primary taste cortex (generally between Y = 10 and Y = 20) (de Araujo et al., 2003c; Grabenhorst and Rolls, 2008; Rolls, 2014a, 2015c). Clear differences between the stimuli as a function of age were not found in this main part of the insular taste cortex (see Figs. 4, 2-factor ANOVA for age × flavor $F_{[10,270]} = 1.1$, $p = 0.35$). This indicates that the differences in the pleasantness of the different beverages as a function of age (shown in Fig. 2) are not related to any simple differences between the age groups in the sensory processing of different flavors in this insular taste cortex region.

In this insular taste cortex region, there was a statistically weak effect of age ($F_{[2,54]} = 3.68$, $p = 0.03$) (relating to the somewhat smaller activations overall found in the Young group as shown in Fig. 4), and an effect of flavor $F_{[5,270]} = 3.89$, $p = 0.002$. In this insular taste cortex region, there were no statistically significant effects of the cognitive labels, as shown by a comparison of the activations to T1 and T2 ($F_{[1,54]} = 3.11$, $p = 0.08$) (see Fig. 4 left).

Further insular cortex activations were found more posteriorly at [−36 −6 10], [36 −6 18], and [38 −4 12] (with a summary in Table S1) in regions where oral texture is represented (de Araujo and Rolls, 2004).

Agranular (anterior) insula cortex

This brain site revealed by taste – rinse (whole brain FWE $p < 0.001$) at [36 26 4] is illustrated in Fig. 5 (right).

The activations extracted at this site to each of the 6 stimuli are illustrated in Fig. 5 (left).

The activations in this brain region are significantly different between the 3 age groups to the set of 6 taste stimuli. This was shown by a two-way ANOVA for the 6 taste stimuli × the 3 age groups performed on the BOLD signal activations, which showed a significant interaction effect ($F_{[10,270]} = 2.34$, $p = 0.01$).

A one-way ANOVA to test for differences of the activations to Fanta (T1) between the age groups showed that there were significant effects, with for example a smaller activation in the Young group to the Fanta (T1) compared to that in the Elderly group ($p = 0.03$).

Overall, it is shown in Fig. 5 (left) that at this brain site, the activations produced to the different stimuli are especially well separated in the Young group. In the Young group, the V8 produces the largest activation (Fig. 5 right), and in the Young group the V8 was unpleasant

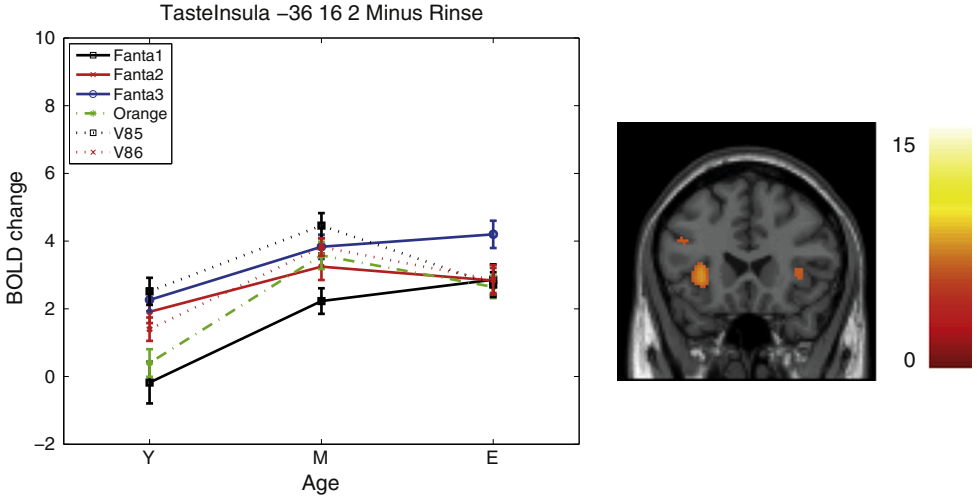

Fig. 4. Right. Activations from the insular taste cortex site at [−36 16 2] identified by a BOLD signal significant to Taste – Rinse (whole brain corrected FWE $p < 0.001$). The activation, shown on the left of the brain, extends 8 mm behind this. The colored bars in the figures indicate the values of the *F* or *t* statistic. Left. The activations at this site shown as the mean change in the BOLD signal, with the error estimate. There was a statistically weak effect of age ($F_{[2,54]} = 3.68$, $p = 0.03$), and an effect of flavor $F_{[5,270]} = 3.89$, $p = 0.002$. There was no significant difference between the age groups in the activations to the different flavors (2-factor ANOVA for age × flavor $F_{[10,270]} = 1.1$, $p = 0.35$).

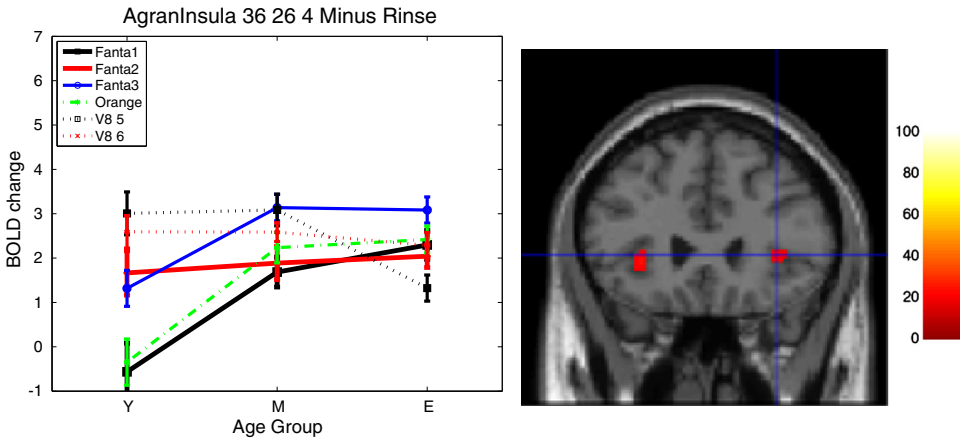

Fig. 5. Activations from the agranular insula site identified by a BOLD signal significant to Taste – Rinse. Right. The brain section shows the agranular insula site [36 26 4] at the cross-hairs (whole brain corrected FWE $p < 0.001$). Left. The activations are the mean change in the BOLD signal, with the error estimate.

(Fig. 2). Fanta T1 and Orange juice (T4), which are well liked by the Young group, produce the smallest activation. Fanta Zero (T2), and the unlabeled Fanta (T3), produce intermediate activations. Thus in general, greater unpleasantness ratings (Fig. 2) were associated with larger activations in this agranular insular cortex region. This interpretation is supported by the additional finding that in the Young group in particular (and not in the Elderly group), agranular insula activations were indeed correlated with the subjective unpleasantness ratings of the beverages provided during the scanning (peak [44 22 6] $z = 3.04$, $p = 0.02$ FWE svc).

Other brain regions activated by flavor – rinse

The flavor – rinse contrast also revealed activations in the somatosensory cortex ([64 −12 24] $p < 0.001$ and [−60 −16 22] $p = 0.015$), with a tendency for the activations to be larger to the V8 stimuli, probably related to texture, for texture-related activations in this region have been identified previously (Grabenhorst and Rolls, 2014); in the medial striatum ([−10 10 0] and [12 10 0]) (which receives from taste-related areas such as the orbitofrontal cortex (Rolls, 2014a)); and in a dorsal anterior region of the cingulate cortex now termed the anterior midcingulate cortex [−4 12 48] (Rolls, 2015a) (where the activations tended to be higher to the V8) (see Table S1). In none of these sites was there an age × flavor interaction, and the activations to the different flavors within an age group were generally close as illustrated in Fig. 4.

Brain regions where the activations are correlated with the pleasantness ratings of the flavors

These sites were identified by a significant positive correlation in the SPM analysis between the activations and the subjective ratings of pleasantness made to every stimulus during the functional neuroimaging.

Amygdala

The activations from one of these sites, in the amygdala, are illustrated in Fig. 6 ([−16 −8 −30] svc $p = 0.018$). Amygdala activation in this region to the sweet (and pleasant) taste of glucose was identified in a prior investigation (O'Doherty et al., 2001), and in addition neurons with taste response in the primate amygdala have been established in previous investigations (Kadohisa et al., 2005a,b; Karadi et al., 1998).

A two-way ANOVA for the 6 flavor stimuli × the 3 age groups performed on the peak BOLD signal activations showed a significant effect of taste ($F_{[5,270]} = 5.40$, $p < 0.001$) across the three age groups. This indicates that the activations in this brain region are significantly different to the set of 6 taste stimuli. A two-way ANOVA for the Fanta, Orange, and V8 flavor stimuli × the 3 age groups performed on the peak BOLD signal showed a significant interaction effect ($F_{[4,108]} = 3.88$, $p = 0.006$), showing that there were differences in the relative responses to the different flavors in the three age groups in the amygdala.

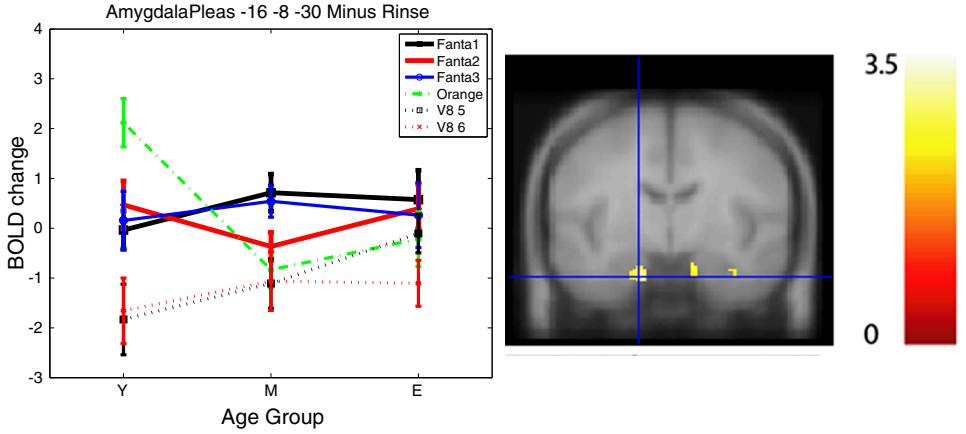

Fig. 6. Right. An amygdala site [−16 −8 −30] where the BOLD signal was positively correlated with the pleasantness ratings (svc $p = 0.018$). The activations are shown on the spm brain average of 305 MRI volumes (avg305T1). The activations at this site to the different stimuli in the different age groups (the mean change in the BOLD signal, with the error estimate).

A one-way ANOVA to test for differences of the activations to the 6 flavors in the Young group showed significant differences ($F_{[5,18]} = 2.82$, $p = 0.02$). Post hoc tests further showed for example that the large activation to orange juice (T4) was significantly different from the activations to Fanta T1 ($p < 0.04$) and Fanta Zero T2 ($p < 0.05$).

A one-way ANOVA to test for differences for the activations to the 6 flavors in the Elderly group showed no significant differences.

These statistical tests show that at this brain site the activations to the different taste stimuli are indeed different in the Young but not in the Elderly group (Fig. 6 left), and that there is a significant flavor × age interaction effect. Overall, at this site, in the Young group there is a wide range of activations related to the pleasantness of the different flavors. The orange juice produces the largest activation, the three Fanta stimuli intermediate activations, and both V8 stimuli the lowest activations. The large activation to the orange juice T4 is consistent with the hypothesis that the "freshly squeezed" orange juice cognitive concept and flavor is something to which this brain value system may be responsive. (At this site, an activation relative to the tasteless solution (the rinse) is related to pleasantness, and a deactivation relative to the tasteless solution is related to unpleasantness.) This interpretation is confirmed by the scatter plots shown in Fig. S1, which show large BOLD signal changes associated with pleasant ratings of the 6 stimuli and small BOLD signals associated with unpleasant ratings. In the Elderly group the activations to the flavors are much more similar to each other. This is consistent with the finding that in general the V8 is more pleasant to the Elderly group and closer to the other stimuli than in the Young group.

Other brain regions where the activations were correlated with the pleasantness ratings

In the orbitofrontal cortex, activations were also found related to the pleasantness ratings of the stimuli ([8 44 −12] $p = 0.032$ svc and [42 44 −14] $p = 0.016$ svc). The activations to the different stimuli were qualitatively similar to those illustrated in Fig. 6, with in particular high activations in the Young group to orange juice (T4) and Fanta Zero (T2), and lower activations to V8 (T5 and T6).

Brain regions where the activations are correlated with the unpleasantness ratings of the flavors:

These sites were identified by a significant negative correlation in the SPM analysis between the activations and the subjective ratings of pleasantness made to every stimulus during the functional neuroimaging.

Dorsal anterior cingulate cortex

The activations from one of these sites, the dorsal cingulate cortex, are illustrated in Fig. 7 ([−6 26 24] svc $p = 0.009$). This is a brain region where the unpleasantness of other stimuli is also represented (Grabenhorst and Rolls, 2011; Rolls, 2014a; Rolls and Grabenhorst, 2008), including in a prior identification the cingulate region activated by monosodium glutamate taste (which is not pleasant (McCabe and Rolls, 2007)) in Fig. 1 of a previous investigation (de Araujo et al., 2003a). This is in a region named the anterior part of the midcingulate cortex (Rolls, 2015a; Vogt, 2009, 2014).

A two-way ANOVA for the 6 taste stimuli × the 3 age groups performed on the peak BOLD signal activations showed a significant effect of flavor ($F_{[5,270]} = 4.10$, $p < 0.001$). This indicates that the activations in this brain region are significantly different to the set of 6 flavor stimuli across the different age groups. A two-way ANOVA for the Fanta, Orange and V8 (T6) flavor stimuli × the Y and E age groups performed on the peak BOLD signal in the cingulate cortex showed a significant interaction effect ($F_{[2,70]} = 3.42$, $p = 0.038$), showing that there were differences in the relative responses to the different flavors in the age groups in the anterior cingulate cortex.

A one-way ANOVA to test for differences of the activations to the 6 flavors in the Young group showed significant differences, for example V8 (T6) > Fanta (T1) $p < 0.01$; V8 (T5) > orange juice (T4) $p < 0.05$; V8 (T6) > orange juice (T4) $p < 0.05$.

A one-way ANOVA to test for differences of the activations to the 6 flavors in the Middle group showed significant differences, for example V8 (T6) > Fanta (T3) $p < 0.02$; V8 (T6) > orange juice (T4) $p < 0.03$; V8 (T5) > orange juice (T4) $p < 0.03$.

A one-way ANOVA to test for differences for the activations to the 6 flavors in the Elderly group showed no significant differences ($F_{[5,90]} = 0.8$, $p = 0.55$).

These statistical tests show that at this brain site the activations to the different flavor stimuli are indeed different in the Young, but not in the Elderly group chosen as a comparison case.

Overall, at this site, Fanta and Orange produce the smallest activation in the Young group compared to the Middle and Elderly groups, i.e. Fanta and Orange in the Young did not activate areas where unpleasantness is represented. The V8 produces a much larger activation than Fanta in the Young group, consistent with the finding that the Young group dislikes the V8. This interpretation is confirmed by the scatter plots shown in Fig. S2, which show large BOLD signal changes associated with unpleasant ratings of the 6 stimuli and small BOLD signals associated with pleasant ratings. In the Elderly group, relatively similar activations are produced to the Fanta and V8 stimuli, consistent with the finding that the Elderly group does not find the V8 unpleasant, as shown in Fig. 2.

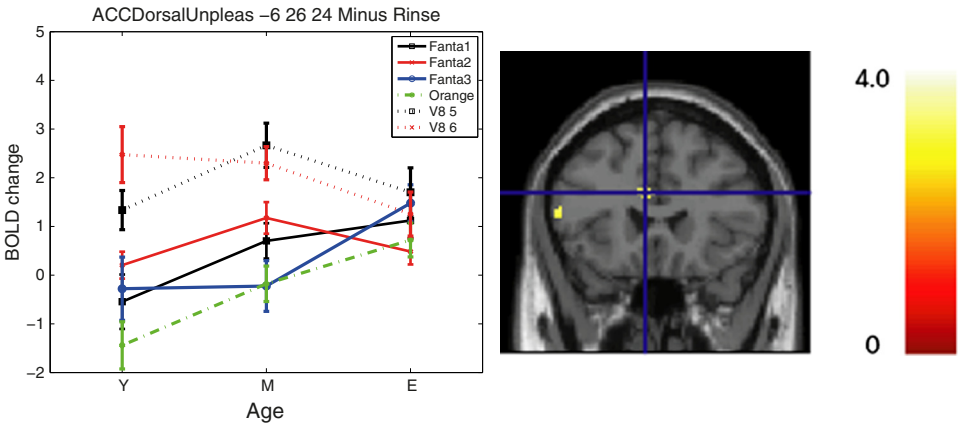

Fig. 7. Right. Activations from the dorsal anterior cingulate cortex site ([−6 26 24] at the crosshairs) where the BOLD signal is correlated with the unpleasantness ratings. The site was identified in the SPM analysis by a significant negative correlation between the BOLD signal and the pleasantness ratings (svc $p = 0.009$). Left. The change in the BOLD signal, with the error estimate, is shown.

Other sites

Similar effects were also found at a lateral opercular cortex site ([−58 22 14] $p = 0.012$ svc), which is lateral to the primary taste cortex, with high activations in the Young group to V8, and low activations to Orange (T4), with intermediate activations to Fanta (T1–T3).

Discussion

This is the first study (to the best of the authors' knowledge) to investigate how the effects produced in different brain areas by complex food stimuli with taste, olfactory, texture and visual components are related to the liking for those foods in subjects grouped into Young, Middle, and Elderly age subsets, and to investigate possible age-related differences in the effects of cognitive descriptive labels of the foods.

In the insular (primary) taste cortex, there were no interactions between age and the activations produced by the stimuli (Fig. 4). Thus differences between the age groups in their liking for and preferences for the different flavor stimuli were not related to relatively different activations to the different stimuli in the insular taste cortex. Consistent with this, activations in the insular taste cortex are related to the intensity of tastes, but not to their pleasantness (Grabenhorst and Rolls, 2008). Further, cognitive descriptions of the flavor stimuli designed to influence preferences did not influence the activations in the insular taste cortex (cf. Grabenhorst et al. (2008)). In addition, the difference in pleasantness for the vegetable flavor across different age groups was much greater than the difference of intensity (Figs. 2 and 3), suggesting that changes in sensitivity to taste and odor with aging are not the main driver of the altered palatability of the foods. Further evidence for the considerable separation of hedonic responses to food from their intensity and identity in primates including humans (Rolls, 2014a) is that when fed to satiety the pleasantness of a food decreases to zero with little change in intensity (Rolls et al., 1983); that neuronal activity and activations in the insular primary taste cortex are related to the concentration of tastes and their intensity but not their pleasantness (Grabenhorst and Rolls, 2008; Rolls et al., 1988; Yaxley et al., 1988); that activations in the pyriform primary olfactory cortex are related to intensity but not pleasantness (Rolls et al., 2008); that neuronal activity and activations in the orbitofrontal cortex (secondary taste cortex) and anterior cingulate cortex are related to the pleasantness but not intensity of the taste and odor of food (Grabenhorst and Rolls, 2008; Kringelbach et al., 2003; Rolls, 2012b, 2014a; Rolls et al., 1988, 1989, 2008; Scott et al., 2005; Scott and Plata-Salaman, 1999; Yaxley et al., 1988); and that chocolate cravers have the same activation to chocolate in the insula, but increased activation relative to non-cravers in the orbitofrontal cortex and anterior cingulate cortex (Rolls and McCabe, 2007). Further, in this investigation, brain areas where activations were related to pleasantness ratings made on every trial did not have activations correlated with the intensity rating made on every trial, providing further evidence for the separation of brain system involved in pleasantness vs intensity. (On average across flavors the activation to the flavors in the insular taste cortex was somewhat smaller in the Young group than in the other two groups (Fig. 4), but that was not a hypothesis of interest in the current study, though it may be of interest in relation to possible effects of age on BOLD signal reactivity across different ages.)

More anteriorly in the agranular insular cortex, a region that receives taste and olfactory inputs from their primary cortical areas and combines these to produce a representation of flavor (de Araujo et al., 2003c), age-related differences were found. In particular, for the agranular insular cortex region identified by the contrast taste – rinse, the Young group showed smaller activations to orange juice and the Fanta stimuli than to the V8 tomato-flavor vegetable juice (Fig. 5). The activations in this area were thus related to the unpleasantness of the stimuli in the Young, which was high in the Young for the vegetable juice, and low (pleasant) for the orange and Fanta. This is supported by the statistically significant correlation between the unpleasantness ratings and the activations at this site in the Young group. Moreover, there was a trend for cognitive effects in this brain region in the Young, in that the activation to Fanta labeled as regular (T1) was lower than to Fanta labeled as Fanta Zero—no added calories (T2) and to unlabeled Fanta (T3), even though the actual Fanta product and flavor being delivered into the mouth were identical (see Fig. 5, though these effects did not quite reach statistical significance).

In brain regions where the activations were correlated with the pleasantness ratings of the food stimuli, such as the amygdala site illustrated in Fig. 6, larger differences were found between the food stimuli in the Young group, with the vegetable juice that was unpleasant in the Young producing especially small effects relative to the other flavors. Interestingly, the orange juice T4 produced an especially large activation here in the Young, perhaps reflecting a property of the fresh orange juice that reflected its healthy, and thus attractive, label “freshly squeezed”. This was not evident in the pleasantness rating (Fig. 2), suggesting that brain activations may reveal more than can be provided by a pleasantness rating. Cognitive effects were suggested in this brain region in the Elderly, in whom the vegetable juice labeled with information about its vitamin and mineral nutrient content (T5) produced a greater activation than just the label “100% vegetable juice” (see Fig. 6, though this effect did not quite reach statistical significance).

In brain regions where the activations were correlated with the unpleasantness ratings of the food stimuli, such as the antero-dorsal cingulate cortex site illustrated in Fig. 7, differences were found between the food stimuli in the different age groups, with the orange juice and Fanta stimuli producing much lower activations in the Young than the Elderly group relative to the vegetable juice, with an intermediate separation in the Middle group (Fig. 7). The ratings of unpleasantness shown in Fig. 2 appear to reflect quite closely the activations found in this cingulate cortex region. This is a part of the cingulate cortex that is designated at the anterior part of the midcingulate cortex (MCC) (Rolls, 2014b; Vogt, 2009, 2014), where the unpleasantness of many other stimuli is represented (Grabenhorst and Rolls, 2011; Rolls, 2014a).

One issue that is raised by the findings is whether the brain activations reflect the pleasantness ratings, or provide the basis causally for the ratings of pleasantness. The latter is likely, that the brain activations at some of the sites described lead to the pleasantness ratings of the foods by the different age groups, for brain function provides the mechanism by which hedonics and behavior are produced (Rolls, 2008b, 2012a, 2014a). (Some of the factors that influence the operation of the brain mechanisms are noted below.) Consistent with the brain regions described here producing the differing hedonics in the different age groups, all the brain regions described here are among the brain regions that are in the known taste, olfactory, and oral texture processing pathways. In particular, the insular primary taste cortex and the pyriform primary olfactory cortex project to the amygdala and orbitofrontal cortex in part by the agranular insula (Rolls, 2014a; Rolls and Grabenhorst, 2008). The orbitofrontal cortex then projects to the anterior cingulate cortex, which can be considered as a tertiary taste cortical area (Grabenhorst and Rolls, 2011; Rolls, 2008a, 2014a).

In some previous investigations, effects of pure taste stimuli on activations have been measured in the elderly (Green et al., 2011, 2013; Jacobson et al., 2010), but the effects reported are not easy to compare with the present findings, because a tasteless control was not used so taste effects as distinct from other effects of the stimuli are difficult to separate; because a rinse of water was used, which is itself known to activate a population of neurons in taste cortical areas (Rolls et al., 1990; Scott et al., 1986; Yaxley et al., 1990) and to produce activations in these areas in humans (de Araujo et al., 2003b) so does not provide a satisfactory baseline reference condition; and because effects of the taste stimuli were reported in those studies in many brain areas believed to have no role in taste processing perhaps for the reasons noted.

It is of interest that brain sites may reflect more information about the stimuli than can be captured by a pleasantness subjective report, as is suggested by the activations evident in Figs. 5–7, and this study

thus provides a foundation for examining how different properties of foods, including cognitive labels, may influence food pleasantness, food choice, food intake, and food consumption. However, significant age × cognitive interaction effects were not found in the analyses performed, so we do not emphasize differences of cognition in the different age groups in this discussion. For example, although the difference in the BOLD response in the amygdala to the V8 T5 labeled as having potential nutritive benefits for the elderly was in the predicted direction (Fig. 6), it was not statistically significant at $p < 0.05$. The smaller effect of cognition in this study than in some previous studies (de Araujo et al., 2005; Grabenhorst et al., 2008) may be related to the fact that instead of providing cognitive descriptors about pleasantness, in this case the advice was less direct, and was cast in terms of potential nutritive benefits, which may appeal differently to different individuals. [In terms of food consumption, soft drinks such as Fanta are more likely to be consumed by younger people, and tomato juice (and V8 is 86% tomato juice) appears to be a product largely avoided until adulthood. In general, tomato juice consumption increases with age, particularly for men. Men over 39 represent 18% of the population and consume 36% of tomato juice. Men and women over age 60 were 16% of the population but consumed 27% of tomato juice (Lucier et al., 2000).] This adds to the considerable evidence that effects can be produced by stimuli that are not available for verbal report (Kouider and Dehaene, 2007; Rolls, 2003, 2014a). Of course many processes are involved in the final outcome implemented by brain regions such as those described, including the effects of genetically driven preferences for sweet and fat, and dislike of bitter; experience with foods such as vegetables (de Wild et al., 2014); conditioning of preferences based on energy and nutritional provision; effects of satiety signals; and the decision-making process itself (Birch, 1999; Booth, 1985; Hetherington et al., 2011; Murray et al., 2014; Rolls, 2012b, 2014a; van den Bosch et al., 2014). In this context, it will be important in future to investigate how nutritious savory non-sweet foods can be made more appealing to the young, and how foods with nutrients for the elderly can be made more attractive to them, and how they influence the processing in the brain regions described here. As shown here, foods of these types do influence the brain systems involved in flavor representation and its pleasantness very differently in different age groups.

Limitations of the current study

The use of the tasteless rinse control could be regarded as both a strength and a weakness of this study. A tasteless rinse control was introduced by Rolls and colleagues after they had discovered that there are single neurons in the primary and secondary taste cortex that respond when water is placed in the mouth (Rolls et al., 1990; Scott et al., 1986; Yaxley et al., 1990). Moreover, water in the mouth produces activations in these areas in humans (de Araujo et al., 2003b). This makes water an inadequate control in imaging studies. Rolls designed the tasteless control by defining a solution that had approximately the main ionic components of saliva. The solution Rolls designed is approximately tasteless to most subjects, though if it were important in a particular study, the solution could be adjusted for each subject individually. However, the design of subtracting the tasteless control goes far beyond this, for when a tastant is introduced in the mouth in a neuroimaging study, the solution must be moved round the mouth to obtain physiological stimulation of taste receptors throughout the oral cavity. The somatosensory stimulation produced by introducing a tastant into the mouth needs to be subtracted from the effects of the taste stimulus, as does the tongue movement used to distribute the liquid round the mouth. Subtraction of effects produced by the tasteless control is designed to do all these things. However, it is the case that if no ratings are made about the tasteless solution, as in the present study, then it is possible that the effects elicited might be a little different. This is a possible limitation of the present study. A solution to this is to present the taste rinse as an extra stimulus, and have a rating made of it. The cost of this of course is that this means that one can use one less experimental stimulus, and that is a real cost. So a slight compromise is usually made, as in this study, to use a tasteless control which follows the test stimulus after a delay. And of course some rinse must be applied between stimuli, so there is no cost to making it a tasteless rinse, as in the current study, and subtracting its effects from the effects produced by the test taste stimulus. The recommended procedure is to subtract the mean response across trials to the rinse stimulus, as this minimizes trial to trial variation in the effects of the rinse due to noise in the system (Rolls and Deco, 2010).

Second, we discuss the identification of the amygdala site illustrated in Fig. 6 as amygdala. The coordinates of the peak identified in SPM were [−16 −8 −30]. It has been pointed out that the AAL atlas (Rolls and Tzourio-Mazoyer, 2015; Tzourio-Mazoyer et al., 2002) classifies this as parahippocampal gyrus. It is true that when it is shown on the SPM single subject T1 image the peak appears rather ventral. But to clarify the issue, we show in Fig. 6 the activation superimposed on the average of 305 MRI scans of the human brain (avg305T1 provided with SPM from Evans et al. (1993)), and on that basis parahippocampal gyrus does not look plausible. The AAL atlas is useful for approximate categorization of brain areas, but not for precise decisions about exactly where a particular activation is located. Further, if one looks at Fig. 6, one sees that the peak happens to be in the lower part of the region activated, so that the region activated is more likely to be the amygdala than anything more ventral. Further, the corresponding activation contralaterally at [12 26 −26] could not be in parahippocampal gyrus, but is in a part of the amygdala. Further, the amygdala is a plausible brain region in which to have flavor-related activations, for there are neurons in the primate amygdala that respond to taste and flavor (Kadohisa et al., 2005a,b; Karadi et al., 1998; Rolls, 1981; Sanghera et al., 1979; Scott et al., 1993; Wilson and Rolls, 1985), and activations in the human amygdala are as much produced by rewarding as by unpleasant taste stimuli (O'Doherty et al., 2001). In contrast, neurons in the parahippocampal gyrus and hippocampus can be influenced by flavor stimuli, but mainly in the context that they are being associated with a place for episodic memory (Kesner and Rolls, 2015; Rolls, 2008b, 2015b; Rolls and Xiang, 2005).

Acknowledgments

The research received support from the Oxford Centre for Computational Neuroscience, and from Coca-Cola Research & Development, Brussels, BE. Author contributions: E T Rolls and M Kellerhals (CocaCola), conceived the fundamental research with the aim of investigating whether there are differences in the systems that process taste and flavor in different age groups. E T Rolls designed and performed the investigation, analyzed the data, and prepared the paper. T E Nichols designed and ran the statistical analyses of all the fMRI data, including preprocessing etc. All authors contributed to the final version of the paper. C Pliatsikos is thanked for help with subject recruitment and for running the scanner.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2015.03.065.

References

Bender, G., Veldhuizen, M.G., Meltzer, J.A., Gitelman, D.R., Small, D.M., 2009. Neural correlates of evaluative compared with passive tasting. Eur. J. Neurosci. 30, 327–338.
Birch, L.L., 1999. Development of food preferences. Annu. Rev. Nutr. 19, 41–62.
Booth, D.A., 1985. Food-conditioned eating preferences and aversions with interoceptive elements: learned appetites and satieties. Ann. N. Y. Acad. Sci. 443, 22–37.
Collins, D.L., Neelin, P., Peters, T.M., Evans, A.C., 1994. Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J. Comput. Assist. Tomogr. 18, 192–205.
de Araujo, I.E.T., Rolls, E.T., 2004. The representation in the human brain of food texture and oral fat. J. Neurosci. 24, 3086–3093.
de Araujo, I.E.T., Kringelbach, M.L., Rolls, E.T., Hobden, P., 2003a. The representation of umami taste in the human brain. J. Neurophysiol. 90, 313–319.

de Araujo, I.E.T., Kringelbach, M.L., Rolls, E.T., McGlone, F., 2003b. Human cortical responses to water in the mouth, and the effects of thirst. J. Neurophysiol. 90, 1865–1876.
de Araujo, I.E.T., Rolls, E.T., Kringelbach, M.L., McGlone, F., Phillips, N., 2003c. Taste-olfactory convergence, and the representation of the pleasantness of flavour, in the human brain. Eur. J. Neurosci. 18, 2059–2068.
de Araujo, I.E.T., Rolls, E.T., Velazco, M.I., Margot, C., Cayeux, I., 2005. Cognitive modulation of olfactory processing. Neuron 46, 671–679.
de Wild, V., de Graaf, C., Jager, G., 2014. Efficacy of repeated exposure and flavour-flavour learning as mechanisms to increase preschooler's vegetable intake and acceptance. Pediatr. Obes. http://dx.doi.org/10.1111/ijpo.244.
Deichmann, R., Josephs, O., Hutton, C., Corfield, D.R., Turner, R., 2002. Compensation of susceptibility-induced BOLD sensitivity losses in echo-planar fMRI imaging. NeuroImage 15, 120–135.
Deichmann, R., Gottfried, J.A., Hutton, C., Turner, R., 2003. Optimized EPI for fMRI studies of the orbitofrontal cortex. NeuroImage 19, 430–441.
Evans, A.C., Collins, D.L., Mills, S., Brown, E., Kelly, R., Peters, T.M., 1993. 3D statistical neuroanatomical models from 305 MRI volumes. Nuclear Science Symposium and Medical Imaging Conference, 1993., 1993 IEEE Conference Record. IEEE, pp. 1813–1817.
Friston, K.J., Worsley, K.J., Frackowiak, R.S.J., Mazziotta, J.C., Evans, A.C., 1994. Assessing the significance of focal activations using their spatial extent. Hum. Brain Mapp. 1, 214–220.
Friston, K.J., Glaser, D.E., Henson, R.N., Kiebel, S., Phillips, C., Ashburner, J., 2002. Classical and Bayesian inference in neuroimaging: applications. NeuroImage 16, 484–512.
Ge, T., Feng, J., Grabenhorst, F., Rolls, E.T., 2012. Componential Granger causality, and its application to identifying the source and mechanisms of the top-down biased activation that controls attention to affective vs sensory processing. NeuroImage 59, 1846–1858.
Grabenhorst, F., Rolls, E.T., 2008. Selective attention to affective value alters how the brain processes taste stimuli. Eur. J. Neurosci. 27, 723–729.
Grabenhorst, F., Rolls, E.T., 2010. Attentional modulation of affective vs sensory processing: functional connectivity and a top-down biased activation theory of selective attention. J. Neurophysiol. 104, 1649–1660.
Grabenhorst, F., Rolls, E.T., 2011. Value, pleasure, and choice in the ventral prefrontal cortex. Trends Cogn. Sci. 15, 56–67.
Grabenhorst, F., Rolls, E.T., 2014. The representation of oral fat texture in the human somatosensory cortex. Hum. Brain Mapp. 35, 2521–2530.
Grabenhorst, F., Rolls, E.T., Bilderbeck, A., 2008. How cognition modulates affective responses to taste and flavor: top down influences on the orbitofrontal and pregenual cingulate cortices. Cereb. Cortex 18, 1549–1559.
Grabenhorst, F., D'Souza, A., Parris, B.A., Rolls, E.T., Passingham, R.E., 2010a. A common neural scale for the subjective pleasantness of different primary rewards. NeuroImage 51, 1265–1274.
Grabenhorst, F., Rolls, E.T., Parris, B.A., D'Souza, A., 2010b. How the brain represents the reward value of fat in the mouth. Cereb. Cortex 20, 1082–1091.
Green, E., Jacobson, A., Haase, L., Murphy, C., 2011. Reduced nucleus accumbens and caudate nucleus activation to a pleasant taste is associated with obesity in older adults. Brain Res. 1386, 109–117.
Green, E., Jacobson, A., Haase, L., Murphy, C., 2013. Can age-related CNS taste differences be detected as early as middle age? Evidence from fMRI. Neuroscience 232, 194–203.
Hetherington, M.M., Cecil, J.E., Jackson, D.M., Schwartz, C., 2011. Feeding infants and young children. From guidelines to practice. Appetite 57, 791–795.
Iannilli, E., Singh, P.B., Schuster, B., Gerber, J., Hummel, T., 2012. Taste laterality studied by means of umami and salt stimuli: an fMRI study. NeuroImage 60, 426–435.
Iannilli, E., Bult, J.H., Roudnitzky, N., Gerber, J., de Wijk, R.A., Hummel, T., 2014a. Oral texture influences the neural processing of ortho- and retronasal odors in humans. Brain Res. 1587, 77–87.
Iannilli, E., Noennig, N., Hummel, T., Schoenfeld, A.M., 2014b. Spatio-temporal correlates of taste processing in the human primary gustatory cortex. Neuroscience 273, 92–99.
Jacobson, A., Green, E., Murphy, C., 2010. Age-related functional changes in gustatory and reward processing regions: an fMRI study. NeuroImage 53, 602–610.
Kadohisa, M., Rolls, E.T., Verhagen, J.V., 2005a. Neuronal representations of stimuli in the mouth: the primate insular taste cortex, orbitofrontal cortex, and amygdala. Chem. Senses 30, 401–419.
Kadohisa, M., Rolls, E.T., Verhagen, J.V., 2005b. The primate amygdala: neuronal representations of the viscosity, fat texture, temperature, grittiness and taste of foods. Neuroscience 132, 33–48.
Karadi, Z., Scott, T.R., Oomura, Y., Nishino, H., Aou, S., Lenard, L., 1998. Complex functional attributes of amygdaloid gustatory neurons in the rhesus monkey. Ann. N. Y. Acad. Sci. 855, 488–492.
Kesner, R.P., Rolls, E.T., 2015. A computational theory of hippocampal function, and tests of the theory: new developments. Neurosci. Biobehav. Rev. 48, 92–147.
Kouider, S., Dehaene, S., 2007. Levels of processing during non-conscious perception: a critical review of visual masking. Philos. Trans. R. Soc. Lond. B Biol. Sci. 362, 857–875.
Kriegeskorte, N., Simmons, W.K., Bellgowan, P.S., Baker, C.I., 2009. Circular analysis in systems neuroscience: the dangers of double dipping. Nat. Neurosci. 12, 535–540.
Kringelbach, M.L., Rolls, E.T., 2004. The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. Prog. Neurobiol. 72, 341–372.
Kringelbach, M.L., O'Doherty, J., Rolls, E.T., Andrews, C., 2003. Activation of the human orbitofrontal cortex to a liquid food stimulus is correlated with its subjective pleasantness. Cereb. Cortex 13, 1064–1071.
Lucier, G., Lin, B.H., Allshouse, J., Kantor, L.S., 2000. Factors affecting tomato consumption in the United States. Vegetables and Specialties/VGS 282pp. 26–32.
Lund, T.E., Madsen, K.H., Sidaros, K., Luo, W.L., Nichols, T.E., 2006. Non-white noise in fMRI: does modelling have an impact? NeuroImage 29, 54–66.
Luo, Q., Ge, T., Grabenhorst, F., Feng, J., Rolls, E.T., 2013. Attention-dependent modulation of cortical taste circuits revealed by Granger causality with signal-dependent noise. PLoS Comput. Biol. 9, e1003265.
McCabe, C., Rolls, E.T., 2007. Umami: a delicious flavor formed by convergence of taste and olfactory pathways in the human brain. Eur. J. Neurosci. 25, 1855–1864.
Murphy, C., 1989. Aging and chemosensory perception of and preference for nutritionally significant stimuli. Ann. N. Y. Acad. Sci. 561, 251–266.
Murphy, C., 1993. Nutrition and chemosensory perception in the elderly. Crit. Rev. Food Sci. Nutr. 33, 3–15.
Murphy, C., Schubert, C.R., Cruickshanks, K.J., Klein, B.E., Klein, R., Nondahl, D.M., 2002. Prevalence of olfactory impairment in older adults. JAMA 288, 2307–2312.
Murray, E., Brouwer, S., McCutcheon, R., Harmer, C.J., Cowen, P.J., McCabe, C., 2014. Opposing neural effects of naltrexone on food reward and aversion: implications for the treatment of obesity. Psychopharmacology (Berl) 231, 4323–4335.
Nitschke, J.B., Dixon, G.E., Sarinopoulos, I., Short, S.J., Cohen, J.D., Smith, E.E., Kosslyn, S.M., Rose, R.M., Davidson, R.J., 2006. Altering expectancy dampens neural response to aversive taste in primary taste cortex. Nat. Neurosci. 9, 435–442.
O'Doherty, J., Rolls, E.T., Francis, S., Bowtell, R., McGlone, F., 2001. The representation of pleasant and aversive taste in the human brain. J. Neurophysiol. 85, 1315–1321.
Rolls, E.T., 1981. Responses of amygdaloid neurons in the primate. In: Ben-Ari, Y. (Ed.), The Amygdaloid Complex. Elsevier, Amsterdam, pp. 383–393.
Rolls, B.J., 1999. Do chemosensory changes influence food intake in the elderly? Physiol. Behav. 66, 193–197.
Rolls, E.T., 2003. Consciousness absent and present: a neurophysiological exploration. Prog. Brain Res. 144, 95–106.
Rolls, E.T., 2005. Emotion Explained. Oxford University Press, Oxford.
Rolls, E.T., 2006. Brain mechanisms underlying flavour and appetite. Philos. Trans. R. Soc. Lond. 361, 1123–1136.
Rolls, E.T., 2008a. Functions of the orbitofrontal and pregenual cingulate cortex in taste, olfaction, appetite and emotion. Acta Physiol. Hung. 95, 131–164.
Rolls, E.T., 2008b. Memory, Attention, and Decision-Making: A Unifying Computational Neuroscience Approach. Oxford University Press, Oxford.
Rolls, E.T., 2012a. Neuroculture. On the Implications of Brain Science. Oxford University Press, Oxford.
Rolls, E.T., 2012b. Taste, olfactory, and food texture reward processing in the brain and the control of appetite. Proc. Nutr. Soc. 71, 488–501.
Rolls, E.T., 2014a. Emotion and Decision-Making Explained. Oxford University Press, Oxford.
Rolls, E.T., 2014b. Emotion and decision-making explained: précis. Cortex 59, 185–193.
Rolls, E.T., 2015a. Emotion and decision-making explained: response to commentators. Cortex 62, 203–210.
Rolls, E.T., 2015b. Limbic systems for emotion and for memory, but no single limbic system. Cortex 62, 119–157.
Rolls, E.T., 2015c. Neural integration of taste, smell, oral texture, and visual modalities. In: Doty, R.L. (Ed.), Handbook of Olfaction and Gustation. Wiley, New York, pp. 1029–1049.
Rolls, E.T., 2015d. Taste, flavor, and appetite. In: Toga, A. (Ed.), Brain Mapping: An Encyclopedic Reference. Academic Press (Elsevier), Amsterdam, pp. 573–580.
Rolls, E.T., 2015e. Taste, olfactory, and food reward value processing in the brain. Prog. Neurobiol. http://dx.doi.org/10.1016/j.pneurobio.2015.1003.1002.
Rolls, E.T., Deco, G., 2010. The Noisy Brain: Stochastic Dynamics as a Principle of Brain Function. Oxford University Press, Oxford.
Rolls, E.T., Grabenhorst, F., 2008. The orbitofrontal cortex and beyond: from affect to decision-making. Prog. Neurobiol. 86, 216–244.
Rolls, E.T., McCabe, C., 2007. Enhanced affective brain representations of chocolate in cravers vs non-cravers. Eur. J. Neurosci. 26, 1067–1076.
Rolls, E.T., Tzourio-Mazoyer, N., 2015. Revised nomenclature for the automated anatomical atlas. Neuroimage.
Rolls, E.T., Xiang, J.-Z., 2005. Reward-spatial view representations and learning in the hippocampus. J. Neurosci. 25, 6167–6174.
Rolls, E.T., Rolls, B.J., Rowe, E.A., 1983. Sensory-specific and motivation-specific satiety for the sight and taste of food and water in man. Physiol. Behav. 30, 185–192.
Rolls, E.T., Scott, T.R., Sienkiewicz, Z.J., Yaxley, S., 1988. The responsiveness of neurones in the frontal opercular gustatory cortex of the macaque monkey is independent of hunger. J. Physiol. 397, 1–12.
Rolls, E.T., Sienkiewicz, Z.J., Yaxley, S., 1989. Hunger modulates the responses to gustatory stimuli of single neurons in the caudolateral orbitofrontal cortex of the macaque monkey. Eur. J. Neurosci. 1, 53–60.
Rolls, E.T., Yaxley, S., Sienkiewicz, Z.J., 1990. Gustatory responses of single neurons in the caudolateral orbitofrontal cortex of the macaque monkey. J. Neurophysiol. 64, 1055–1066.
Rolls, E.T., Grabenhorst, F., Margot, C., da Silva, M.A.A.P., Velazco, M.I., 2008. Selective attention to affective value alters how the brain processes olfactory stimuli. J. Cogn. Neurosci. 20, 1815–1826.
Sanghera, M.K., Rolls, E.T., Roper-Hall, A., 1979. Visual responses of neurons in the dorsolateral amygdala of the alert monkey. Exp. Neurol. 63, 610–626.
Scott, T.R., Plata-Salaman, C.R., 1999. Taste in the monkey cortex. Physiol. Behav. 67, 489–511.
Scott, T.R., Yaxley, S., Sienkiewicz, Z.J., Rolls, E.T., 1986. Gustatory responses in the frontal opercular cortex of the alert cynomolgus monkey. J. Neurophysiol. 56, 876–890.
Scott, T.R., Karadi, Z., Oomura, Y., Nishino, H., Plata-Salaman, C.R., Lenard, L., Giza, B.K., Aou, S., 1993. Gustatory neural coding in the amygdala of the alert macaque monkey. J. Neurophysiol. 69, 1810–1820.
Scott, T.R., Edwards, E.M., Smith, C.A., Hilgert, K.G., Schwartz, G.J., Pritchard, T.C., 2005. Medial orbitofrontal cortex: its role in mediating satiety in the macaque. Chem. Senses 30 (Suppl. 1), i190.

Small, D.M., 2010. Taste representation in the human insula. Brain Struct. Funct. 214, 551–561.
Small, D.M., Prescott, J., 2005. Odor/taste integration and the perception of flavor. Exp. Brain Res. 166, 345–357.
Small, D.M., Zald, D.H., Jones-Gotman, M., Zatorre, R.J., Pardo, J.V., Frey, S., Petrides, M., 1999. Human cortical gustatory areas: a review of functional neuroimaging data. Neuroreport 10, 7–14.
Small, D.M., Gregory, M.D., Mak, Y.E., Gitelman, D., Mesulam, M.M., Parrish, T., 2003. Dissociation of neural representation of intensity and affective valuation in human gustation. Neuron 39, 701–711.
Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., Niazy, R.K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J.M., Matthews, P.M., 2004. Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage 23 (Suppl. 1), S208–S219.
Stevens, J.C., Cruz, L.A., Hoffman, J.M., Patterson, M.Q., 1995. Taste sensitivity and aging: high incidence of decline revealed by repeated threshold measures. Chem. Senses 20, 451–459.
Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. NeuroImage 15, 273–289.
van den Bosch, I., Dalenberg, J.R., Renken, R., van Langeveld, A.W., Smeets, P.A., Griffioen-Roose, S., Ter Horst, G.J., de Graaf, C., Boesveldt, S., 2014. To like or not to like: neural substrates of subjective flavor preferences. Behav. Brain Res. 269, 128–137.
Veldhuizen, M.G., Albrecht, J., Zelano, C., Boesveldt, S., Breslin, P., Lundstrom, J.N., 2011. Identification of human gustatory cortex by activation likelihood estimation. Hum. Brain Mapp. 32, 2256–2266.
Vogt, B.A. (Ed.), 2009. Cingulate Neurobiology and Disease. Oxford University Press, Oxford.
Vogt, B.A., 2014. Submodalities of emotion in the context of cingulate subregions. Cortex 59, 197–202.
Weiskopf, N., Hutton, C., Josephs, O., Deichmann, R., 2006. Optimal EPI parameters for reduction of susceptibility-induced BOLD sensitivity losses: a whole-brain analysis at 3 T and 1.5 T. NeuroImage 33, 493–504.
Weiskopf, N., Hutton, C., Josephs, O., Turner, R., Deichmann, R., 2007. Optimized EPI for fMRI studies of the orbitofrontal cortex: compensation of susceptibility-induced gradients in the readout direction. MAGMA 20, 39–49.
Wilson, F.A.W., Rolls, E.T., 1985. Reinforcement-related unit activity in basal forebrain and amygdala. Soc. Neurosci. Abstr. 11, 525.
Yaxley, S., Rolls, E.T., Sienkiewicz, Z.J., 1988. The responsiveness of neurons in the insular gustatory cortex of the macaque monkey is independent of hunger. Physiol. Behav. 42, 223–229.
Yaxley, S., Rolls, E.T., Sienkiewicz, Z.J., 1990. Gustatory responses of single neurons in the insula of the macaque monkey. J. Neurophysiol. 63, 689–700.