Improved methods and analysis in fMRI studies to assess taste and aroma integration

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Introduction: Perception of flavour is a complex process involving the integration of taste and aroma. Few fMRI studies [1,2] have assessed the cross-modal interactions arising from flavour processing. fMRI studies typically use a retro-nasal aroma delivery and a delayed swallow following taste and aroma delivery. However, a delayed swallow will delay the retro-nasal aroma release [3], and thus considerably diminish a component of taste-aroma integration. A further consideration is the analysis strategy to assess integration. Previously, a supra-additive response to a congruent flavour has been demonstrated [1,2] using a subtraction analysis (combination of taste and aroma (flavour) minus taste minus aroma). However, such a subtraction method would cause those areas responding to mouth and tongue movements to be subtracted twice from the flavour stimulus [4] and these oral somatosensory areas may themselves be involved in flavour integration [5]. Here we compare a unimodal taste and unimodal retro-nasal aroma stimuli with a congruent taste and aroma combination "flavour" and employ an immediate swallow to maximise flavour integration. Conjunction [4] and subtraction analysis strategies and the use of a control stimulus are assessed to study crossmodal taste-aroma interactions.

Materials and Methods: 13 right-handed healthy subjects (7 male and 6 female) took part in the study which was approved by the local Research Ethics Committee, all subjects gave informed written consent.

Paradigm: In each fMRI cycle (**Fig. 1**) we delivered in a random order, over a 3 s period, 3 ml of one of the following: **(i) Unimodal taste (T):** 3% sucrose; **(ii) Unimodal aroma (A):** 0.1 % v/v isoamylacetate (IAA) (which has a banana/pear aroma); **(iii) Congruent flavour stimulus (TA):** a combination of both 3 % sucrose and 1 % IAA. **(IV) Control (C)**: tasteless and aroma-less solution "mock saliva" (distilled water containing trace amount of KCl and NaHCO3) [3]. After each stimulus delivery, two mouth rinses were delivered to clear the oral cavity of lingering taste and aroma compounds using 5 ml of the same control solution over a 5 s period (**rinses**). A visual cue instructed the subjects to swallow immediately after each delivery. 24 cycles were acquired for each subject. The liquid stimuli were administered using an automated spray delivery system as described in [3]. Each stimulus was sprayed gently across the oral cavity to achieve extensive stimulation of the taste receptors through the dispersion of the liquid on the tongue and other mouth surfaces before the swallowing.

Data Acquisition: Images were acquired on a 3 T custom-built Nottingham system using an insert head gradient coil and whole head TEM volume coil. In order to maximize the BOLD signal across brain areas with different tissue T_2* relaxation times, a double echo EPI technique was used. 26 contiguous coronal double-gradient-echo EP images with TE of 22 and 39 ms (1.9 kHz gradient switching frequency, 64x64 matrix, voxel size 4x4x5 mm^3) were acquired from +64 to -66 anterior-posterior MNI coordinate, every 2.6 sec (jittered). Following the fMRI paradigm, a T_2* map of the same 26 coronal slices was created from a multi-echo EPI image set of four echoes, TE = 22, 39, 56 and 73 ms.

Data Analysis fMRI data were processed using SPM2. Slice timing correction was applied to the 1st and 2nd echo EPI images, the 1st echo images were aligned, and motion parameters subsequently applied to the 2nd echo images. Any subject who moved more than one voxel during the fMRI paradigm was excluded from the study. T_2* maps were calculated from the 4 echo data sets using a pixel-by-pixel, linear weighted least squares fit, and used for weighted summation [6] of the two fMRI data sets. The fMRI data were then normalised to the standard SPM2 EPI template. Spatial smoothing at 10x10 x12.5 mm^3 FWHM, global scaling and temporal filtering with a 128 sec high pass filter cut-off were applied. A general linear model was formed with the stimuli (T, A, and TA) and control (C) modelled as a 3 s box function convolved with a canonical HRF with temporal derivative, individual realignment parameters and the two mouth rinses were included as covariances of no interest. To identify the main effect of the cortical response to T, A, TA and C, four contrast vectors were formed for each subject and pooled to form a second level random effects (RFX) group. To assess the effect of the control stimulus, RFX maps were formed for each stimuli subtracted from the control: (T – C), (A – C) and (TA – C) and RFX maps were formed. To investigate the nature of the "Integration of Taste and Aroma" (i) a subtraction method with the contrast vector [(TA – C) – (T – C) – (A – C)] = [TA – (T + A) + C]) was used to identify supra-additive interactions and (ii) a conjunction analysis of (TA - T) and (TA - A) was performed [(TA - T) ∩ (TA - A)]. The (TA - T) contrast reflects areas with a greater response to flavour than to unimodal taste (aroma and taste areas in flavour, i.e, bimodal areas) whilst (TA - A) reflects areas that are greater in response to flavour than unimodal aroma (taste and aroma areas in flavour). Thus a conjunction analysis of these two contrasts should reveal areas that show greater activity to flavour than both unimodal taste and unimodal aroma stimuli alone. A binary mask of all regions showing response to taste, aroma, flavour and control stimuli (P < 0.001, uncorrected) was used in all subtraction analyses.

Results: The control stimulus itself showed widespread activation including the oral somatosensory areas (SI, SII, mid-insula, rolandic operculum); precentral gyrus; ACC and its rostral part with adjoining medial OFC; superior temporal gyrus; inferior, mid, medial and superior frontal gyrus. Activation maps using the subtraction analysis to study the supra-additive response to the flavour are shown in Fig 2 and corresponding maps for the conjunction analysis in Fig 3. Both methods showed similar activation areas including rolandic operculum, anterior cingulate cortex, and medial and caudomedial OFC; inferior frontal gyrus and inferior parietal lobe. The subtraction method revealed activity of insula, frontal operculum, amygdala, and mid- and superior frontal gyrus compared to the conjugate analysis, whilst the conjugate analysis showed additional activation in SII, rostral-, mid- and posterior cingulate and fusiform gyrus compared to the subtraction analysis.

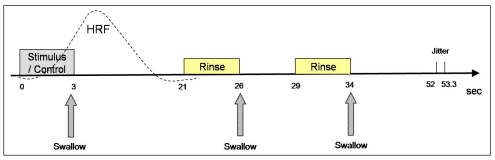

Fig.1 One cycle of fMRI paradigm

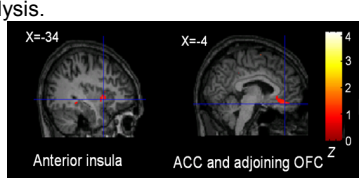

Fig.2 RFX maps for subtraction analysis, p <.05

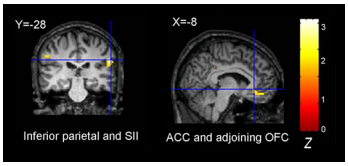

Fig.3 RFX maps for conjunction analysis, p <0.05

Discussion: We have mapped the cortical representation to unimodal taste, unimodal aroma and flavour using an immediate swallow paradigm and a control stimulus to reduce oral somatosensory effects. We show that the control stimulus activated a wide network of brain areas and itself may have different sensory and reward attributes than the taste stimulus under investigation. Comparison taste or retro-nasal aroma stimulus with a control stimulus may therefore cancel out the cortical response under investigation. Subtraction [1,2] and a conjunction analysis [4] shows a supra-additive response to taste and aroma stimuli in OFC, insula, frontal operculum, and ACC which support previously reported activations [1,2]. In addition, activation of the rolandic operculum, inferior frontal gyrus and inferior parietal lobe and oral somatosensory areas (SII) was found using the conjugate analysis, and not shown by subtraction analysis. This finding supports the hypothesis of Small [5] that oral somatomotor areas play the principal role in binding taste, aroma, and oral somatosensory modalities into a unitary flavour percept.

References: [1] De Araujo I.E. et al (2003). Eur. J. Neurosci 18: 2059-68. [2] Small D. J. et al (2004) Neurophysiol 92: 1892-903. [3] Marciani L. et al (2006) J Neurosci Methods 158:186–94. [4] Calvert G. (2001) Cereb Cortex 11:1110-23. [5] Small D.M. (2008) Chem Percept 1:136-46. [6] Posse S et al (1999) MRM 42:87-97. **Acknowledgements:** This work was funded by the BBSRC.