ELSEVIER

Journal of Neuroscience Methods 158 (2006) 186–194

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

Improved methods for fMRI studies of combined taste and aroma stimuli

Luca Marciani [a,b,*], Johann C. Pfeiffer [c], Joanne Hort [c], Kay Head [b], Debbie Bush [d], Andy J. Taylor [c], Robin C. Spiller [a], Sue Francis [b], Penny A. Gowland [b]

[a] *Wolfson Digestive Diseases Centre, University Hospital, University of Nottingham, Nottingham NG7 2UH, UK*
[b] *Sir Peter Mansfield Magnetic Resonance Centre, University of Nottingham, UK*
[c] *Flavour Research Group, Division of Food Sciences, University of Nottingham, UK*
[d] *Surgery, University Hospital, University of Nottingham, UK*

Received 30 March 2006; received in revised form 26 May 2006; accepted 27 May 2006

Abstract

Previous neuroimaging studies of the cortical representation of gustatory and olfactory stimuli have often delivered tastants to the mouth in very small quantities or stimulated olfaction orthonasally. In studies of retro-nasal olfaction, swallowing was generally delayed to reduce head motion artefacts. The present fMRI study aims to improve upon such methodological limitations to allow investigation of the cortical representation of flavour (taste and aroma combination) as it typically occurs during the consumption of liquid foods. For this purpose we used (1) a novel, automated, sprayed stimulus delivery system and a larger volume of liquid sample (containing sweet tastants and banana/pear aroma volatiles) to achieve more extensive stimulation of the oral cavity taste receptors, (2) a *pseudo-natural delivery* paradigm that included prompt swallowing after each sample delivery to obtain physiological retro-nasal olfactory stimulation, (3) fMRI acquisition with wide brain coverage and double-echo EPI to improve sensitivity. We validated our paradigm for the delivery of volatiles using atmospheric pressure chemical ionisation mass spectrometry. This showed that the main retro-nasal delivery of volatiles in the paradigm occurs immediately after the swallow. Several brain areas were found to be activated, including the insula, frontal operculum, rolandic operculum/parietal lobe, piriform, dorsolateral prefrontal cortex, anterior cingulate cortex, ventro-medial thalamus, hippocampus and medial orbitofrontal cortex.

Keywords: Gustatory; Olfactory; Flavour; Retro-nasal; Oral; APcI-MS; Echo-planar imaging

1. Introduction

Human brain mapping techniques have made it possible to define human cortical representations of gustatory and olfactory stimuli. This has allowed the investigation of both primary sensory (Berthoud, 2002; Drewnowski, 1997; French and Cecil, 2001; Rolls, 1997; Rolls and Rolls, 1997; Rowland et al., 1996; Schwartz et al., 2000) and secondary perceptual interactions (Calvert, 2001; Delwiche, 2003; Hort and Hollowood, 2004), which are ultimately important in the control of feeding (Rolls, 1997; Schwartz et al., 2000). The anterior insula and adjoining frontal operculum form the primary taste cortex (Small et al., 1997, 1999). The rolandic operculum in the parietal cortex has recently also been implicated to be part of the primary taste cortex (Faurion et al., 1999; Ogawa et al., 2005). Secondary projections have been reported in the caudal orbitofrontal cortex (OFC) (Francis et al., 1999), amygdala (O'Doherty et al., 2001), anterior cingulate (Small et al., 2003), ventral striatum (O'Doherty et al., 2003) and dorsolateral prefrontal cortex (Kringelbach et al., 2004). Olfactory inputs are processed in the primary olfactory cortex located in the piriform cortex (Cerf-Ducastel and Murphy, 2001; Zatorre et al., 1992) as well as in a far anterior region of the insula and in the amygdala (Cerf-Ducastel and Murphy, 2001; O'Doherty et al., 2000). The OFC (Rolls, 2004a,b) and ventral striatum (Berns et al., 2001; O'Doherty et al., 2002) receive projections from these areas, respond to aroma (O'Doherty et al., 2000; Zatorre et al., 1992) and appear to show differential activity to pleasantness and reward (Rolls, 2000).

Abbreviations: AC, anterior cingulated; APcI-MS, atmospheric pressure chemical ionisation mass spectrometry; BOLD, blood oxygenation level dependent; EPI, echo-planar imaging; fMRI, functional magnetic resonance imaging; FWHM, full width at half maximum; HRF, Haemodynamic response function; MNI, Montreal Neurological Institute; OFC, orbitofrontal cortex; PET, positron emission tomography; TE, echo time; TTP, time to peak

* Corresponding author. Tel.: +44 115 9514747; fax: +44 115 9515166.
E-mail address: Luca.Marciani@nottingham.ac.uk (L. Marciani).

0165-0270/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2006.05.035

More recently, studies have moved towards investigating interactions and association effects between taste and aroma (Djordjevic et al., 2004; Hort and Hollowood, 2004; Small et al., 1997, 2004; Taylor and Hort, 2004). The sensory responses to food characteristics (taste, aroma, texture, temperature) and the perceptual interactions between such stimuli are thought to combine to determine the perceived flavour of food (Taylor, 1996; Taylor and Linforth, 1996, 1999). For this study we define taste as the perception associated with a tastant delivered to the mouth, and aroma as the perception associated with volatiles delivered retro-nasally during swallowing (as opposed to volatiles delivered orthonasally during sniffing), and flavour is defined as the perception of a taste/aroma combination, neglecting at this stage other known factors such as temperature or texture. Neural pathways for taste (Gautier et al., 1999; O'Doherty et al., 2001, 2000; Small et al., 1999; Zald et al., 2002) and aroma (Cerf-Ducastel and Murphy, 2001; Rolls, 2000; Zatorre et al., 1992) stimuli have been shown to converge on the anterior, agranular insula cortex, the anterior OFC, amygdala and anterior cingulate (AC) (De Araujo et al., 2003a). These are the areas where the neural representation of flavour is thought to be formed.

Functional magnetic resonance imaging (fMRI) provides a unique tool with which to study the cortical pathways of taste and aroma and the resulting cortical interactions and association effects. Experiments aimed at studying these cortical interaction and association effects must mimic realistic food consumption conditions as closely as possible. This is difficult in fMRI experiments that require the subject to lie supine and still, within an acoustically noisy MRI scanner. There have been a number of limitations in the fMRI paradigms used to date to investigate the response to taste and aroma. Firstly, previous studies have only delivered very small volumes (typically 50–75 μl) of tastant to the mouth, often to a specific part of the tongue, either quickly (De Araujo and Rolls, 2004; De Araujo et al., 2003a; Kringelbach et al., 2004; O'Doherty et al., 2001) or over a period of a few seconds (Small et al., 2004). A more realistic paradigm would feed larger volumes (Frank et al., 2003; Gautier et al., 1999), and would expose more of the oral cavity receptors.

Secondly, most previous studies have prevented the subject from swallowing immediately after stimulus delivery, to avoid contamination of the fMRI response to taste with movement resulting from swallowing. However, holding a foodstuff in the mouth for an extended period will affect its perception, particularly when lying supine. More importantly, delayed swallowing delays the main retro-nasal delivery of aroma volatiles that occurs with physiological swallowing, as will be shown in this study using sensory laboratory techniques. This is an important aspect of flavour perception, as swallowing carries volatiles from the back of the oral cavity, via the nasopharynx, to the olfactory receptors (Buettner et al., 2001). Neuroimaging studies of olfaction have generally stimulated the ortho-nasal pathway, presenting aromas to the nose using swabs (Koizuka et al., 1994; Levy et al., 1997; Small et al., 1997; Zatorre et al., 1992) or air flow direct to the nostrils, sometimes using olfactometric systems (Francis et al., 1999; Kobal and Kettenmann, 2000; Lorig et al., 1999; O'Doherty et al., 2000; Sobel et al., 1998; Yousem et al., 1997; Zald and Pardo, 1997).

Ideally, a study of association of taste and retro-nasal aroma should allow natural swallowing after tastant delivery. Swallows should also be cued to allow physiological and reproducible retro-nasal stimulation in conjunction with the taste stimulation. In this study we cued the subjects to swallow immediately at the end of the taste stimulus delivery. We term this the "*pseudo-natural delivery*" protocol throughout this paper. We are aware of one PET study that has not delayed swallowing after stimulus delivery (Gautier et al., 1999), whilst other fMRI studies of taste and aroma interactions have simply asked subjects to swallow "regularly as needed" without precise control of the timing (Cerf-Ducastel and Murphy, 2001, 2003, 2004).

Finally, many previous fMRI studies have focused scanning to limited number of thin slices on the OFC and insula region to increase temporal resolution and improve local shimming. Furthermore, a single echo time has been usually chosen as a compromise between the need to minimize signal drop-out (shorter TE) in the OFC whilst maintaining sensitivity to the blood oxygenation level dependent (BOLD) effect (TE = T_2^*) across other brain regions such as the insula, where T_2^* is longer. In this study we have widened brain coverage to encompass the parietal lobe as part of the primary taste cortex. We have also used a double-echo technique to improve BOLD sensitivity across brain areas with a range of T_2^* values.

In summary the primary aim of this study was to improve methods for fMRI studies of the cortical representation of flavour during relatively normal consumption of liquid foods, using:

(a) A novel, automated stimulus delivery system, delivering 3 ml of each stimulus as a spray across the oral cavity, to achieve extensive stimulation of the taste receptors.
(b) A *pseudo-natural delivery* paradigm that cued swallowing immediately at the end of each sample delivery to cause physiological, retro-nasal, olfactory stimulation, as validated using sensory laboratory techniques.
(c) A MRI acquisition with wide brain coverage and using double-echo EPI for improved BOLD sensitivity.

2. Methods

2.1. Subjects

Twelve healthy right-handed subjects (seven males and five females, between 25 and 36 years of age, average body mass index 24 kg/m^2 and range 20–27 kg/m^2) took part in the fMRI study (Section 2.4). The volunteers were not asked to fast overnight, but all experiments were carried out at least 2 h after eating. The local Medical School Research Ethics Committee approved this study and all subjects gave informed written consent before attending the scanning session. Six healthy volunteers (four males and two females, between 26 and 36 years of age, average body mass index 24 kg/m^2 and range 22–27 kg/m^2) participated in the paradigm validation experiment (Section 2.5). This procedure was covered by separate Ethics approval and volunteers consented to participate.