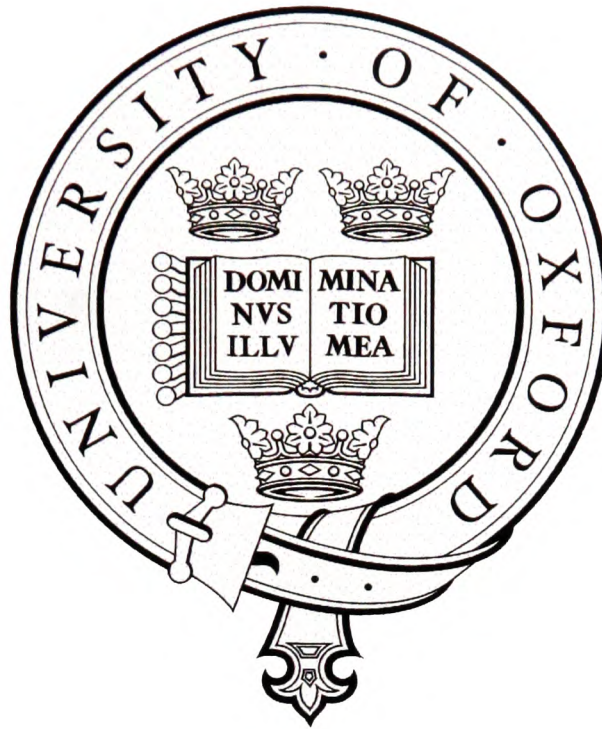


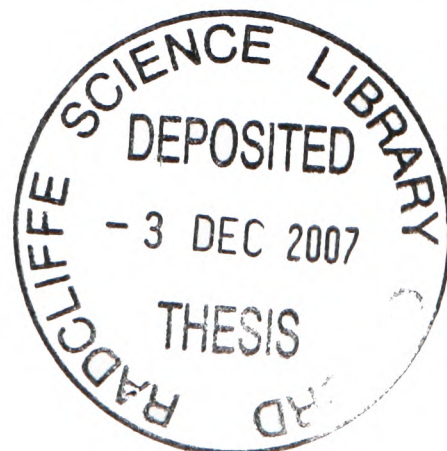


MULTISENSORY INTEGRATION OF OLFACTION

Robert Alexander Österbauer

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Oxford.

Christ Church College



Trinity Term 2006

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ABSTRACT

The primary aim of this thesis was to investigate, using functional magnetic resonance imaging (fMRI), the neurophysiological basis of multisensory integration involving smell and vision. To achieve this goal, several technical challenges had to be addressed: the attainment of sufficiently high quality fMRI images in olfactory brain regions within the orbitofrontal cortex (OFC), the construction of a stimulus delivery system adequate for rapid and controlled odour delivery in the MRI environment, and optimal strategies for delivering and perceiving liquid flavour stimuli in the scanner.

In two initial fMRI experiments, strategies including sensitivity encoding and passive shimming to improve OFC image quality were explored. The results demonstrated that both methods can improve signal detection in OFC, a brain area particularly sensitive to susceptibility artefacts. In a further fMRI study, the effectiveness of two methods of delivering odorants dissolved in liquids was compared. In this study, the same set of participants was required to either swallow the liquid immediately after delivery or hold it in their mouths for a brief period of time. The results indicated that while both methods allowed detection of activity in primary olfactory and gustatory cortices, activation of the OFC was not observed when participants swallowed the liquids immediately. This was presumed to be due to the increased head motion associated with swallowing.

Finally, the mechanisms underlying visual-olfactory integration were investigated using a combination of behavioural and imaging methods. An initial behavioural study revealed strong colour-odour associations for certain smells associated with fruits (e.g. lemon – yellow). In a subsequent fMRI study, volunteers were presented with a selection of the most colour-associated odours from the prior behavioural study either in isolation or in the presence of congruent and incongruent colours. Analysis of the fMRI data revealed that a highly left lateralised network of brain areas comprising of the OFC and insular showed increasingly stronger responses to odour-colour combinations of higher congruency. In a follow-up fMRI study, this same network was also found to be responsible for integrating odours, not only with colours, but also with their corresponding visual images (objects). In sum, the series of fMRI studies undertaken in this thesis argue for a fundamental role of the OFC in the integration of olfactory-visual inputs in the human brain.

This thesis contains approximately 50,000 words.

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1 Introduction

The following chapter provides an overview of what is currently known about the anatomy and physiology of the human olfactory system. The workings of this sensory system are discussed from the level of olfactory receptors that interact with odour molecules, to the cortical organisation of olfactory pathways, with a particular emphasis on findings provided by the neuroimaging technique of functional magnetic resonance imaging (fMRI). Additionally, findings from a variety of behavioural studies presenting evidence for multisensory interactions between the olfactory and visual system are discussed. From this, a variety of hypotheses are derived about the physiological mechanisms of olfactory-visual integration which are investigated in later chapters and are the main subject of this thesis.

1.1 INTRODUCTION

The ability to detect airborne chemicals (odorants) plays an important role in virtually all vertebrate and invertebrate organisms by influencing feeding and mating behaviour (Herz and Cahill 1997) as well as allowing for the identification of other members of the same species and aiding navigation (Lushekin and Shuleikina 1989; Streng and Wallraff 1992). The detection of molecules is considered the ontogenetically oldest sensory modality and, interestingly, shows a high degree of functional similarity in phylogenetically diverse organisms (Ache 1994). The mammalian olfactory system is capable of perceiving and discriminating an immense

variety of odorant molecules of differing shapes and sizes that may themselves only be present in extremely low concentrations. It is estimated that humans are able to detect more than ten thousand different odorants, that they can discriminate among as many as five thousand and that the detection threshold might be as low as fifty odour molecules. Despite the clear importance of olfaction, this remains one of the least well studied sensory systems, particularly in humans.

Over the past decade, progress has been made in elucidating the mechanisms of sensory transduction. However, our understanding of the information coding principles occurring at higher stages in the olfactory system remains sparse. At the level of olfactory receptors and bulb, most of our knowledge has been derived from studies in lower vertebrates, primarily mice and rats, whereas cortical processing of olfaction has typically been studied in primates including humans. The anatomy and physiology of the human olfactory system is therefore described in the first part of this chapter.

Since the advent of neuroimaging techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), a few studies have begun to investigate the central mechanisms of the human olfactory system. These will be reviewed in the second part of this chapter.

In our natural surroundings, odours are frequently accompanied by visual stimuli from where the odours emanate. The strength of the associations between objects and their odours is reflected in the fact that virtually all odours are named after the particular object they are associated with (e.g. the smell of oranges). This is one obvious distinction between olfaction and the other sensory modalities (e.g. the colour blue may be associated with a vast amount of different objects). From everyday experiences, such as eating and drinking, as well as from a variety of behavioural

experiments, it is known that the visual properties of an object may influence the perception of its odour. The final part of this chapter gives a review of the behavioural evidence for these interactions between the visual and olfactory system and proposes a neural mechanism that ultimately gives rise to these perceptions.

1.2 ORGANISATION OF THE OLFACTORY SYSTEM

1.2.1 Olfactory receptor neurones and epithelium

The olfactory receptor neurones (ORNs) are embedded in the olfactory epithelium that lines the posterior nasal cavity in humans (see Figure 1.1). The epithelium contains the bipolar ORNs, supporting cells and olfactory stem cells that are capable of proliferation and differentiation into ORNs. Because the olfactory receptor cells only have a life span of 30-60 days, they are continually replaced from a basal layer of stem cells (Calof et al. 1991).

Each ORN extends 10-30 cilia (containing the signal transducing receptors) into the olfactory mucus that covers the epithelium and projects an axon to the ipsilateral olfactory bulb of the brain. The mucus is secreted by both supporting cells and Bowman's glands and is thought to provide an appropriate environment for changes in ciliary membrane potential and for the diffusion of the usually lipophilic odour molecules (Getchell et al. 1984). The mucus is rich in odour binding proteins (OBPs), small (ca. 20 kDa) soluble proteins that belong to a large family of ligand carrier proteins called lipocalins (Pelosi 1998). The various OBPs are specialised to bind to different structural classes of odorants. Even though the precise function of the OBPs is still not determined, they might enhance the odour response by concentrating

odour molecules in the mucus, presenting them to the ORNs and facilitating their inactivation and removal. Therefore the most likely role of the OBP is to transport specific volatile compounds across the mucus and present these to the olfactory receptor neurones. Additionally, OBPs appear to be involved in the pre-selection of volatile compounds that are biologically relevant and ultimately interact with the olfactory sensory cells. This indicates a role of OBPs as a specific filter and not only as a passive shuttle protein for odorants in the mucus layer of the olfactory epithelium (Lobel et al. 2002). However, there is no evidence that the OBPs are directly involved in the binding between odorant and receptor.

The transduction process takes place at specialised odorant receptors (ORs) that are distributed over the surface of the cilia. In mice, a large multigene family appears to code approximately 1000 different OR that are selectively expressed by ORNs (Buck and Axel 1991). Because both the basic organisation of the olfactory system and the structure of the ORNs show a high degree of similarity between mice and humans, these findings are likely to apply to humans as well. The ORs are G-protein coupled receptors with seven transmembrane domains. There is evidence that a specific ORN only expresses a single type of receptor, which would make the ORN sensitive to either a single odorant or to a single structural aspect of various odour molecules (Ressler et al. 1994). The exact molecular mechanisms of receptor-odorant interaction are still unknown, but at present, the most widely accepted explanation argues for direct binding between odorant and receptor and therefore for encoding of the structural aspects of odorants. Because most ORNs respond to a variety of different odorants (Firestein et al. 1993), it is unlikely that the perceived odour quality results from activation at the level of single receptors that is then conveyed to the olfactory cortex without further processing. A more plausible scheme is one in which

the combined activity of a variety of ORNs, each encoding a single structural feature of the odour molecule, gives rise to perceived odour quality.

Activation of the OR leads to a series of second messenger events, which ultimately depolarise the ORN from its normal resting potential of 70-85mV and leads to the generation of an action potential (Schild and Restrepo 1998) that is relayed to the olfactory bulb. In most cases, the OR is coupled to a G-protein (Golf) that initiates a cascade of second messengers. The amplification produced by second messenger cascades greatly enhances the sensitivity of the ORN. In fact, some studies suggest that the transduction cascade initiated by a single odour molecule might be sufficient to generate an action potential in the ORN (Menini et al. 1995). This would make detection thresholds of the olfactory system comparable to the visual system in the sense that quantal events (single molecules) can be detected and signalled by the nervous system.

Although the olfactory system has no strict topographical organisation like other sensory modalities, the distribution of olfactory receptors in the olfactory epithelium already shows a very rough spatial organisation. The existence of functionally different zones and hence differential sensitivity to odours in the rat olfactory epithelium has been studied with the electro-olfactogram (EOG) and with voltage sensitive dyes. Using these techniques, it was shown that even though odorants can usually stimulate all regions in the epithelium, the region of peak responsiveness differs for different odours, which indicates a coarse topographic organisation at the level of the epithelium (Mackay-Sim and Kesteven 1994). Moreover, the location of the most responsive site for a given odorant was similar in all animals studied and hence appears to be well preserved.

In situ hybridisation studies have also shown that ORNs expressing the same olfactory receptor are broadly distributed on the epithelium, but only within certain regions (Ressler et al. 1993). This study demonstrated in mice that this differential expression of ORNs results in at least four distinguishable spatial zones within the epithelium which contain neurones with the same olfactory receptors. The zones themselves consist of a series of longitudinal stripes along the anterior-posterior axis of the nasal cavity. Using EOG, it has also been shown that the physiological response to different odours varied systematically in a fashion parallel to the four gene expression zones (Scott et al. 1997). This could indicate that the ORNs grouped in one zone might share common receptive properties.

1.2.2 Olfactory bulb

Despite the possibility that some qualitative aspects of odours may already be coded at the level of the olfactory epithelium, the bulk of signal processing appears to occur in the olfactory bulb - the first site of synaptic processing in the mammalian olfactory system. Clearly, extensive processing needs to take place in order to achieve the discrimination ability of the human olfactory system, as the quantity of functionally distinct human olfactory receptors -estimated at around 350 (Malnic et al. 2004) - is considerably lower than the 10,000 or so individual odours that can be discriminated. The majority of these encoding processes appear to take place in the olfactory bulb, the first relay station in the olfactory pathway.

On the surface of the olfactory bulb the axons of ORNs form synapses within specialised, roughly spherical, bundles of dendritic processes called glomeruli (Morrison and Costanzo 1992). Individual glomeruli receive inputs from ORNs

originating in all regions of the olfactory epithelium which precludes a strict topographic organisation characteristic of other sensory modalities. Instead, ORNs that are dispersed throughout the epithelium converge on small regions of the bulb. There is however evidence that the glomeruli are functional units with different odours mapped onto different glomeruli (Bozza and Kauer 1998; Mori et al. 2006). This suggests that ORN afferents can segregate by odorant responsiveness and that local regions of the olfactory bulb may receive inputs from ORNs with similar response properties. In the glomerulus, the axons of the ORNs synapse with the dendrites of inhibitory interneurons (periglomerular cells), mitral cells and tufted cells (Fig 1.1).

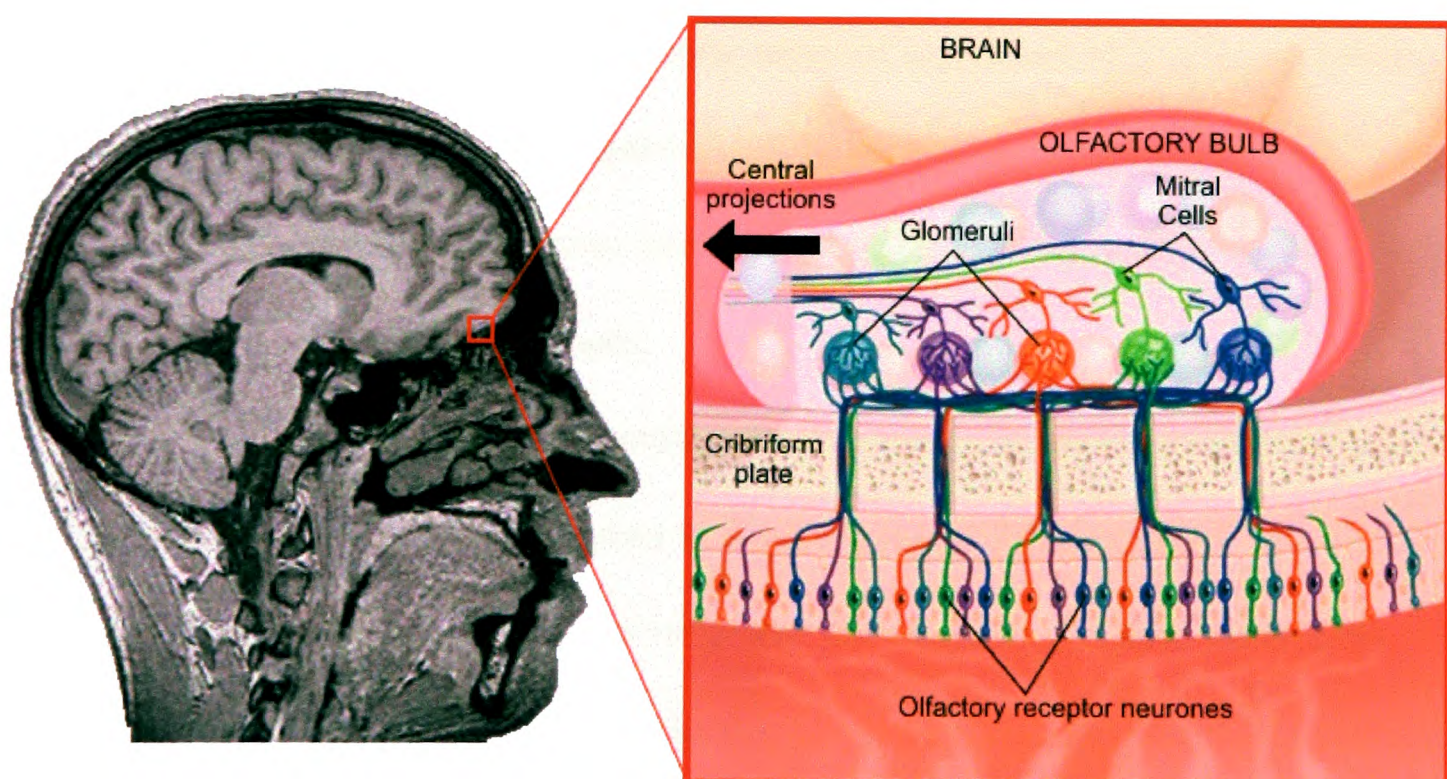


Figure 1.1: Olfactory bulb.

The approximate location of the olfactory bulb in humans (red box) is shown on a sagittal MR image of the author. The right inset shows a schematic illustration of the olfactory epithelium and the olfactory bulb. Four different olfactory receptor neurones (each depicted in a different colour) with converging projections to different glomeruli within the olfactory bulb are shown. The axons of the mitral cells form the lateral olfactory tract which projects to piriform cortex, anterior olfactory nucleus, amygdala and entorhinal cortex. [Bulb illustration modified from Brooks/Cole, Thomson Learning, 2001]

Additionally, the excitatory mitral and tufted cells form reciprocal dendro-dendritic synapses with inhibitory granule cells. Mitral cells have richly arborised dendritic structures and axon collaterals that give rise to feed-forward and feed-back circuits within the olfactory bulb (Chuah and Zheng 1992).

A further refinement in the processing of olfactory information encoded in the glomeruli appears to be the result of the inhibitory granule cells. The granule cells receive excitatory inputs from the mitral and tufted cells (M/T) and in turn send inhibitory projections to neighbouring M/T cells and this connectivity probably gives rise to a mechanism of lateral inhibition (Yokoi et al. 1995). According to this model, the glomeruli in a circumscribed region of the olfactory bulb respond to a relatively wide variety of structurally related odour molecules, albeit with different firing rates, and inhibit neighbouring M/T cells. The inhibition of neighbouring M/T cells then results in the 'sharpening' of the molecular range of individual M/T cells, narrowing down the initially quite broad receptive field. In addition to an increased specificity, this mechanism would also increase sensitivity by suppressing random noise generated by neighbouring neurones.

The mitral cells are the principal output neurones in the olfactory bulb and the axons of the M/T cells merge together to form the lateral olfactory tract. The radiating fibres of the olfactory tract further diverge into the lateral and medial striae at the olfactory trigone that send parallel projections to several regions. The fibres of the medial olfactory striae terminate for the most part in the parolfactory area, whereas some project to the subcallosal gyrus and a further subset extends to the anterior perforated substance. The larger lateral olfactory striae send parallel projections to five different areas of the olfactory cortex: the anterior olfactory nucleus that connects the two olfactory bulbs, the primary olfactory (piriform) cortex, the olfactory tubercle

from where a projection goes to the medial dorsal nucleus of the thalamus, the cortical nucleus of the amygdala that projects to the hypothalamus, and the entorhinal cortex that projects to the hippocampus.

1.2.3 Central projections from the olfactory bulb

As mentioned above, projections from the olfactory bulb reach several distinct regions in the human brain. Prominent amongst these is the piriform cortex. Located at the junction of the frontal and temporal lobes, and considered primary olfactory cortex in primates, including humans (Tanabe et al. 1975) piriform cortex is the largest cortical recipient of afferent fibres from the olfactory bulb (Figure 1.2). At this point it is important to mention the unique organization of the olfactory system, which does not have a thalamic relay between sensory receptor and cortex, unlike all other sensory systems (Sherman and Guillery 1996). Since the publication of the first neuroimaging study on human olfaction (Zatorre et al. 1992), several subsequent studies have implicated the piriform cortex in various aspects of odour perception and have demonstrated the central role of this brain region in olfactory processing including in the perception of odour intensity (Anderson et al. 2003), valence (Gottfried et al. 2002a), familiarity (Plailly et al. 2005), odorant structure (Gottfried et al. 2006) and odour habituation (Poellinger et al. 2001).

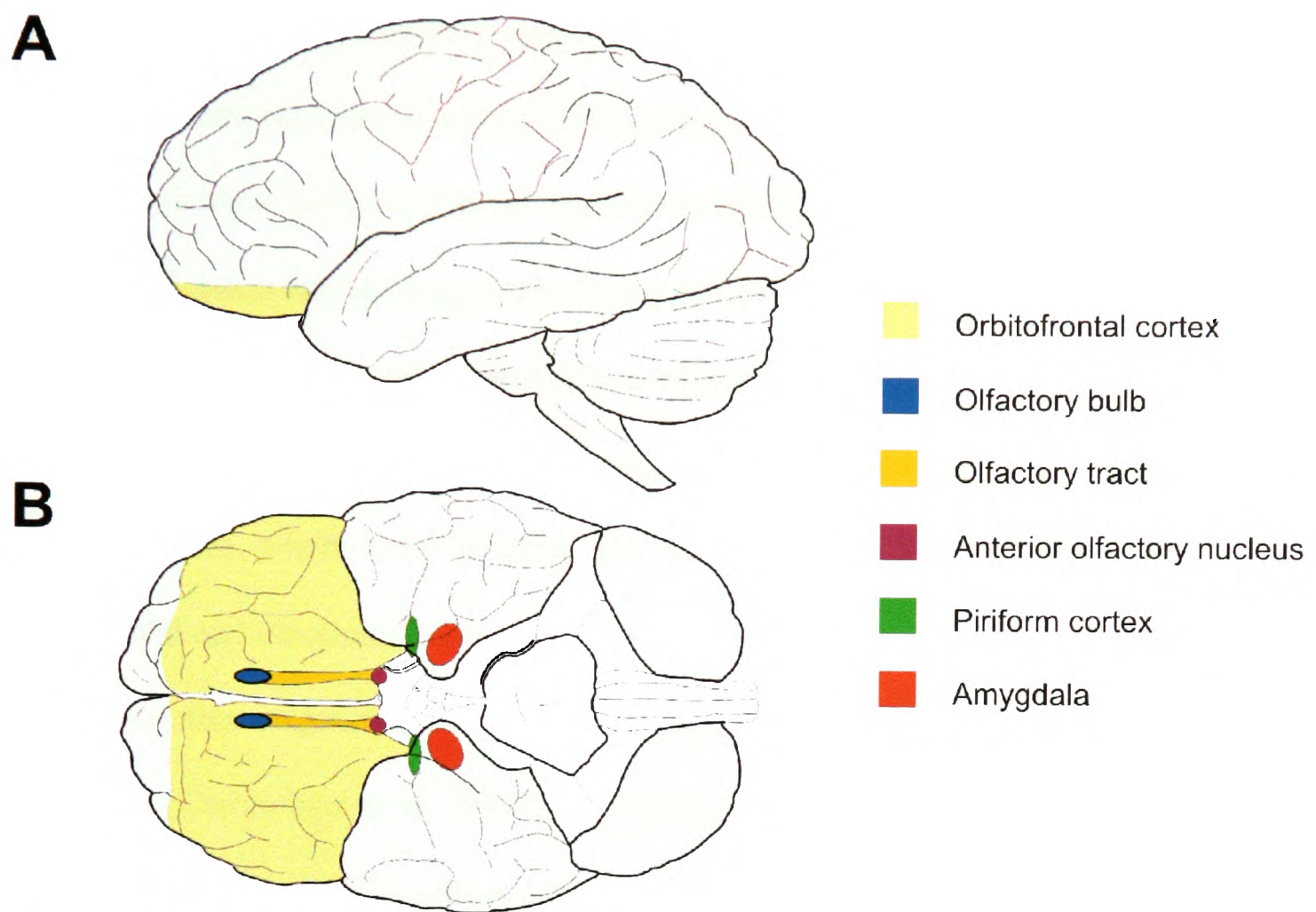


Figure 1.2: Central olfactory pathways.

A The location of the orbitofrontal cortex, shown on a sagittal view of the human brain.

B Ventral view of the human brain, depicting the major recipients of olfactory signals originating from the olfactory receptor neurones.

The piriform cortex extends projections to several other brain structures including the amygdala, mediodorsal thalamus, hypothalamus, insula cortex, hippocampus and orbitofrontal cortex (OFC). Most prominent amongst these regions is the OFC, corresponding to secondary olfactory cortex in primates, including humans (Carmichael et al. 1994; Rolls 2001; Tanabe et al. 1975).

A second region receiving inputs from the olfactory bulb is the anterior olfactory nucleus (AON) which is located between the olfactory bulb and piriform cortex. Little is known about its role in human olfaction, but it appears to be involved in the feedforward regulation of information passing from the bulb to the piriform

cortex and in the feedback regulation of the return circuit (Brunjes et al. 2005). Moreover, it relays information between the left and right olfactory bulbs via the anterior commissure and serves a similar role in distributing information to the left and right piriform cortices. The precise role of this structure remains poorly understood, but it has been suggested that it receives olfactory input from individual glomeruli and combines the information to recreate representations of complex odour stimuli (Haberly 2001). The AON then feeds this assembled information forward to piriform cortex, where it can be integrated into an analysis that includes access to multimodal and emotional cues.

Another major recipient of direct inputs from the olfactory bulb is the amygdala (Carmichael et al. 1994), in particular the anterior cortical nucleus and medial nucleus, both of which are reciprocally connected to the piriform cortex. While some studies have argued that the amygdala is specifically involved in the processing of aversive odours (Gottfried et al. 2003; Zald and Pardo 1997), others have claimed that odour intensity, rather than valence, is encoded in this region (Anderson et al. 2003). In a more recent study (Winston et al. 2005) it was shown that the amygdala exhibits an intensity-by-valence interaction, with differential responses to high-intensity odours (compared to low-intensity odours) for pleasant and unpleasant smells, but not neutral ones. The authors concluded that the amygdala encodes neither intensity nor valence per se, but rather a combination of both which in turn reflects the overall emotional value of the odour.

Another central projection from both the olfactory bulb and piriform cortex reaches the entorhinal cortex, which in turn is extensively connected to the hippocampus (Carmichael et al. 1994; Murray et al. 2005). This pathway appears to be

mainly involved in the formation and retrieval of odour memories (Cerf-Ducastel and Murphy 2006; Gottfried et al. 2004).

1.2.4 Orbitofrontal cortex

The human OFC, which lines the ventral surface of the frontal lobes, is the major recipient of olfactory inputs from primary olfactory regions described above and is considered the most important secondary olfactory cortex (Carmichael et al. 1994; Gottfried and Zald 2005; Ongur and Price 2000; Rolls 2001). The OFC receives direct input from primary olfactory cortex, even though the majority of inputs to the OFC are relayed via the mediodorsal thalamus. In its anatomy, the OFC shows considerable sulcal variability in humans, but three main patterns have been recently described (Kringelbach and Rolls 2004). Because the seminal cytoarchitectonic map of the human brain generated by Brodmann does not show the OFC in much detail, recent attempts have been made to rectify this situation (Ongur et al. 2003). Based on these more detailed maps, the OFC can be separated into 5 distinct areas (10, 11, 12, 13 and 14), which themselves have further subdivisions (Figure 1.3).

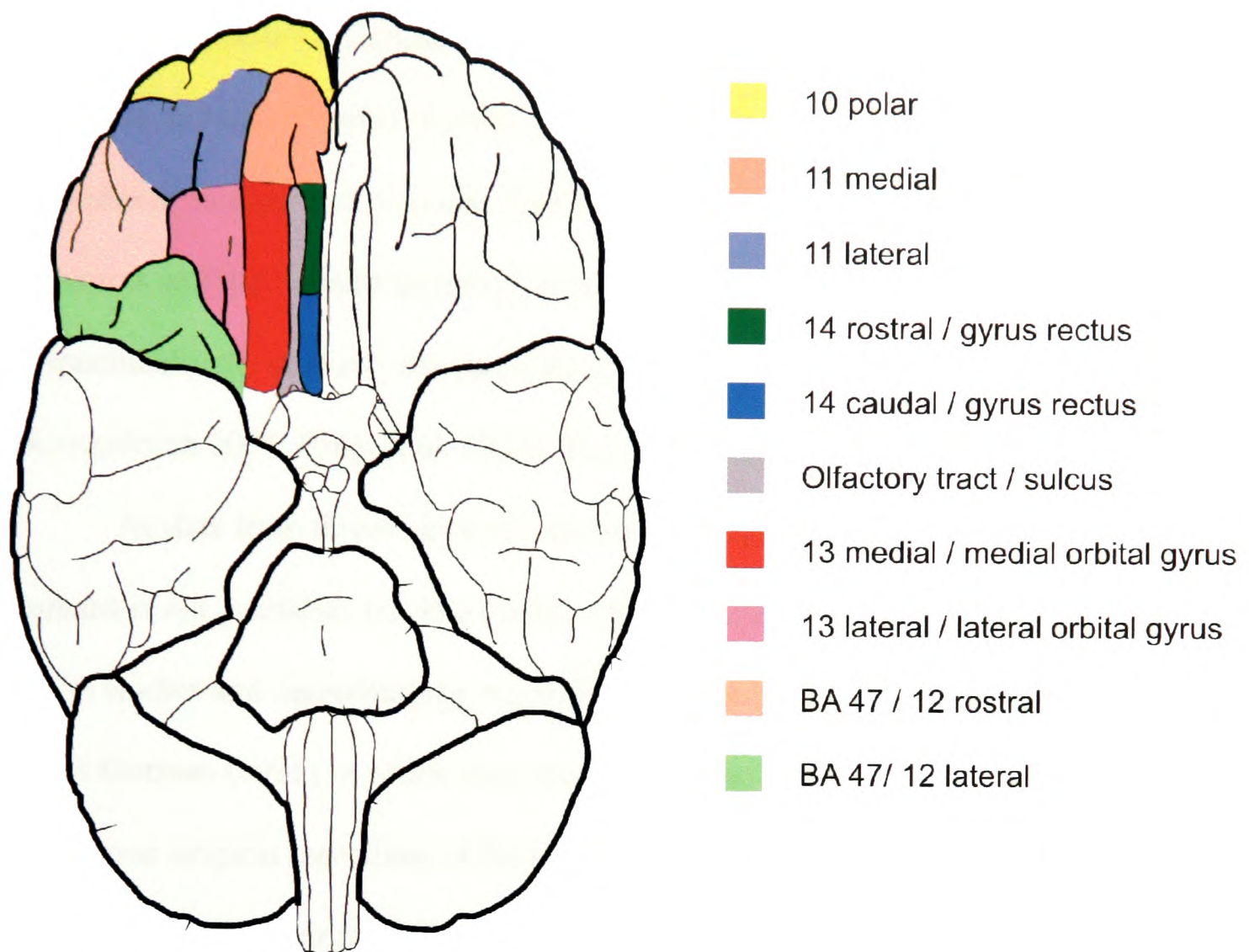


Figure 1.3: Anatomy of the OFC.

Subdivisions of the human OFC shown on a ventral view of the human brain. The regional separations are based on those described by Ongur and colleagues (2003)

Based on studies conducted in monkeys (Tanabe et al. 1975), one would expect the olfactory regions in the human OFC to be approximately located in the medial and lateral aspects of area 13. These areas are, however, extensively interconnected with all other regions in the OFC, which are the recipients of sensory information from a variety of modalities, including the visual, somatosensory, visceral and gustatory system (Ongur and Price 2000). In particular, the interaction between the olfactory and gustatory system in the OFC appears to be the main determinant of flavour perception during the consummation of food and drink. In addition to

receiving inputs from a variety of modalities, the OFC is also heavily interconnected with limbic structures (Carmichael and Price 1995a), including the cingulate cortex, amygdala and insula cortex. Further connections are formed within the nucleus accumbens, caudate and putamen. The connections between the OFC and limbic structures and the 'reward circuitry' in the ventral striatum has led to the notion that it is specifically the positive affect of pleasant odours that are encoded here (Kringelbach 2005; Rolls et al. 2003a; Royet et al. 2000).

As data from direct electrode recordings during olfactory stimulation in humans is not available, our knowledge about OFC function is primarily derived from lesion studies and neuroimaging experiments. In a seminal study by Zatorre and Jones-Gotman (1991) in which they investigated the performance of patients that had undergone surgical resections of the OFC, it was found that whilst odour detection remained intact, odour identification and discrimination were disrupted. These deficits were particularly pronounced in patients with resections in the right hemisphere, leading the authors to hypothesise that there is a hemispheric specialisation for olfactory processing. This notion was further substantiated when the same researchers conducted the first human neuroimaging study on olfaction and found that a set of 8 different odorants activated the right OFC much more strongly than the left (Zatorre et al. 1992). Subsequent studies have, however, not been consistent in demonstrating a right hemispheric dominance for odour perception and it appears that effects of laterality are dependent on the type of olfactory task performed (Royet and Plailly 2004).

1.3 NEUROIMAGING OF THE OLFACTORY SYSTEM

To date, less than 200 neuroimaging studies (fMRI and PET) have concentrated on the function of the human olfactory system. This relatively meagre number of studies by comparison to the number conducted on other sensory modalities, is probably due to the technical difficulties associated with the controlled presentation of odours. The following section provides an overview of the neuroimaging studies that have so far been concerned with the neural mechanisms involved in basic olfactory processing (see the following chapter for a description of the technical aspects of fMRI) such as odour intensity, familiarity and pleasantness perception.

At this point it is important to give a definition of the various olfactory perceptions that are employed in the present thesis. By odour detection, I mean the conscious perception of the presence of an odour, irrespective of any other judgement. Clearly, this constitutes the lowest stage in the cognitive processing of an odour. Related to odour detection is the concept of odour intensity perception, which quantifies the amount of odour being detected and therefore relates to the physical concentration of the odour. In general, the perceived magnitude of odour intensity grows as a power function of the physical concentration, similar to intensity perception in other sensory systems. Typically, the perceptual intensity of odours is measured on some form of rating scale.

In addition to this quantitative aspect of odour perception, two more qualitative aspects are frequently reported in the literature, namely familiarity and pleasantness. Familiarity is an entirely subjective concept relating to whether an odour has previously been encountered by a person and may therefore vary tremendously across the population for a given odour. Familiarity constitutes the lowest level of

odour recognition. Odour identification on the other hand is the highest level in the odour recognition process since it refers to the ability to correctly name the odour (e.g. 'the smell of strawberries').

The terms odour pleasantness/hedonics/valence are used interchangeably in this thesis and refer to the emotional response to an odour which can vary on a negative to positive continuum, ranging from highly aversive/disgusting to highly pleasant/appetitive. Again, pleasantness perception is highly subjective and the same odorant may be perceived as pleasant by some people and as unpleasant by others. Related to this is the observation that expectations about odours may strongly influence odour hedonics. This power of expectations has recently been demonstrated in an fMRI study where the same odour cheesy smelling odour was either labelled as 'cheddar cheese' or 'body odour' (de Araujo et al. 2005). Participants in this study rated the odour as significantly more unpleasant when it was labelled as 'body odour'.

It is important to mention here that most odours do not exclusively stimulate the olfactory nerve alone (pure olfactants), but rather simultaneously stimulate both the olfactory and trigeminal nerve. This trigeminal stimulation is responsible for the tactile (burning, cooling) and irritating component of odour perception which is a quality of all pungent smells. Even though the trigeminal system is separate from the olfactory system, during the subjective experience of an odour it is very difficult to dissociate the olfactory from the trigeminal aspects of a scent, a strong example of multisensory integration involving olfaction (Cain and Murphy 1980). Odours vary greatly in the degree to which they stimulate the trigeminal nerve, but in most cases this aspect is negligible (Doty et al. 1978). Therefore, the fMRI investigations mentioned below do not explicitly state whether the odour stimuli had any trigeminal component. Accordingly, the trigeminal component of odours used for the work

presented in this thesis has not been explicitly measured, because its influence on visual-olfactory integration was considered insignificant. However, trigeminally irritating odours may elicit immediate avoidance responses on the basis of their trigeminal component and therefore trigeminal components have a strong influence on odour hedonics. Such a relation between trigeminal components and unpleasantness is most likely due to the fact that many toxic substances are strongly trigeminal.

In the absence of any specific task other than detection, odours appear to activate both the primary olfactory cortex (POC), consisting of piriform cortex and adjoining amygdaloid complex, and OFC (Dade et al. 1998; Poellinger et al. 2001; Small et al. 1997; Sobel et al. 2000; Zatorre et al. 1992). Several of these studies suggest that it is odour intensity that is encoded at the level of the POC (Poellinger et al. 2001; Rolls et al. 2003a; Sobel et al. 2000). Indeed, many of these studies have demonstrated that the POC responds with increasingly higher amplitude to higher concentrations of odorants and relays this information to the OFC. While this would suggest that POC activation should therefore be observable in virtually any olfactory study, this has not always proven to be the case (Yousem et al. 1999; Zald and Pardo 1997).

The absence of POC activation in some studies may be attributable to two factors: habituation and sniffing. Firstly, the olfactory system habituates rapidly to continuous stimulation. For example, a recent fMRI investigation found that the initial increase in POC activity decreased to baseline within 30 s of continuous stimulation (Sobel et al. 2000). Additionally, the height of the BOLD response was found to decrease over that course of the experiment.

In a further fMRI investigation comparing either 9s or 60s of odour stimulation, Poellinger and colleagues (2001) found that the POC displayed a brief

increase in the BOLD signal for about 10s, followed by a prolonged decrease below baseline. Therefore, the prolonged olfactory stimulation of 90seconds or more as employed in some imaging studies may have caused the response in POC to habituate which could explain the absence of POC activation. Conversely, the OFC showed a sustained increase in activation which lasted for the duration of the odour presentation. Such observations would suggest that the duration of odour presentations in fMRI investigations ought to be kept to a minimum.

A second reason for the absence of POC activation in some studies may lie in the fact that sniffing odourless air in itself, activates these regions (Sobel et al. 1998a). Since most studies employ an experimental design in which subjects are cued to inhale through their nose while odours are delivered to them, this might affect activation in POC, in particular when an odourless control condition is subtracted from the odour trials.

In contrast to the POC, activation in OFC in response to odours is consistently reported. Indeed, in their meta-analysis of 13 different neuroimaging studies that all used pleasant odours and did not involve a cognitive task other than odour detection, Gottfried and Zald (2005) found consistent activation around the lateral area 13 of the OFC, suggesting that this is the main region of olfactory association cortex.

Olfactory judgements of familiarity have been intensely investigated by Royet and co-workers (Plailly et al. 2005; Royet et al. 2001; Royet et al. 1999). In their first published PET study (1999), they found that odour familiarity judgments were mainly associated with activity of the right OFC, the subcallosal gyrus, left inferior and superior frontal gyrus and the anterior cingulate. In a subsequent PET study (2001), subjects were required to make various odour judgments (detection, intensity, hedonicity, familiarity and edibility) on a range of different odorants. Whilst all five

olfactory tasks induced activation in the right OFC, the observed activity in this region was highest during familiarity judgments and lowest during the detection task. The authors concluded that various aspects of odour processing in the OFC are lateralized and that odour recognition and judgements of familiarity are primarily processed in the right OFC.

Further evidence of a right hemispheric lateralisation for odour recognition and familiarity comes from a recent fMRI study by the same research group (Plailly et al. 2005). Participants in this study were exposed to a large variety (57) of odours and were required to either detect the presence of an odour or judge its familiarity. In contrast to their previous results, activity in the right OFC was not found to differ between the two tasks. However, activation particularly in the right POC (but also in right hippocampus and left inferior frontal gyrus and amygdala) was higher during familiarity judgements, indicating that odour familiarity may already be encoded at the level of the piriform cortex. The failure to observe differences within the OFC may be attributable in part to the different imaging modalities used (PET vs. fMRI), but clearly further work is required before any firm conclusions may be drawn.

Considering the hedonic primacy of odours, it is not surprising that numerous studies have focused on odour pleasantness/aversiveness (de Araujo et al. 2003c; Gottfried et al. 2002c; 2003; O'Doherty et al. 2003; O'Doherty et al. 2000; Rolls et al. 2003a; Royet et al. 2000; Savic et al. 2002; Winston et al. 2005; Zald and Pardo 1997; Zatorre et al. 2000), which appears to be encoded primarily within the medial OFC. This is consistent with the observation that the OFC is involved in hedonic processing across a variety of sensory modalities, including olfaction (Kringelbach 2005).

There is currently some controversy over the role of the amygdala in the processing of odour hedonics, since the established view that this region particularly

encodes aversive odours (Royet et al. 2000; Zald and Pardo 1997) has been challenged by several more recent studies. For example, Anderson and colleagues (2003) used the pleasant odour citral (lemon smell) and the unpleasant odour valeric acid (rancid smell) in high and low concentrations so that they could vary the valence and intensity of these stimuli. They found that amygdala activation was associated with odour intensity, but independent of valence. Conversely, activity in the OFC was associated with odour valence, independent of intensity. In a similar study using more odours (3 pleasant and 3 unpleasant), Rolls and co-workers (2003a) found that ratings of odour intensity were correlated with the height of the blood oxygenation level dependent (BOLD) signal in medial olfactory cortical areas (including the piriform and anterior entorhinal cortex), but not in the OFC. In contrast, pleasant odours were found to activate a medial region of the OFC, whereas unpleasant odours activated the left lateral OFC, irrespective of intensity.

A more recent fMRI study (Winston et al. 2005) on the role of the amygdala in hedonic odour processing also included two hedonically neutral odours in addition to a pleasant and unpleasant one and presented all stimuli at high and low concentrations. The results showed that the responses in amygdala correlated with odour intensity for pleasant and unpleasant smells but not for neutral smells. Conversely, when odour hedonics were varied (at constant intensity), the amygdala was preferentially activated by pleasant and unpleasant odours but not by neutral ones. These findings suggest that the role of the amygdala is more complex than simply encoding representations of intensity rather than emotional valence. Instead, amygdala activity appears to reflect intensity only for emotionally salient (positive or negative) odours, thereby generating a representation of the overall emotional value of a stimulus.

1.4 INTEGRATION OF OLFACTION AND VISION

In our natural surroundings, it is seldom the case that odours are perceived in isolation. Rather, odours are frequently accompanied by gustatory and visual cues that interact with, and modulate, our subjective experience of the stimuli from which they emanate. Despite the relevance of such interactions across many perceptual and cognitive domains, the multisensory integration of olfaction and vision has received comparatively less attention than that between the visual, auditory and tactile modalities (Calvert et al. 2004; Welch and Warren 1986), presumably because of difficulties associated with controlled stimulus presentation of olfactory stimuli. In this section, the behavioural findings on multisensory integration is discussed, followed by a review of the hypothesized neural mechanisms responsible for these behavioural effects.

The majority of behavioural studies that have investigated the influence of visual cues on olfactory perception in humans have been conducted in the context of food, i.e. on flavour perception. At this point it is important to mention that our perception of the foods and beverages we consume typically involves the integration of multiple sensory inputs relating to the taste, smell, look, texture, temperature and trigeminal attributes of a product (see Verhagen and Engelen (2006) for an excellent review of multisensory interactions involving food). Together, these multisensory interactions play a critical role in determining a unified flavour percept and also highly influence the perceived pleasantness of the foodstuffs we eat. Nevertheless, the relative contribution of olfaction to the perceived flavour appears to be quite substantial, an effect that everyone with a blocked nose due to a cold will be aware of. In such conditions, food tastes extremely bland. Experimentally, it has been shown

that up to 80 percent of the overall reported 'taste' of a solution disappears if the nose is blocked (Murphy et al. 1977).

To allow accurate comprehension of the remainder of this section, several of the terms used in the context of olfaction will now be defined. The term flavour encompasses the perceived tastes (sweet, salty, sour, bitter and umami) and odours, which may be perceived both by sniffing and retronasally while consuming foods (Burdach and Doty 1987; Burdach et al. 1984; Rozin 1982) of a stimulus, for the moment ignoring trigeminal irritation, temperature and tactile components which also contribute to the overall sensation of flavour (Forde et al. 2002; Green 1993; Green et al. 2005; Lawless and Stevens 1984). Whilst the flavour percept is undoubtedly influenced by all these sensory interactions, this thesis will focus primarily on the influence of vision on olfactory perception. Therefore, this section is specifically concerned with the role that visual cues may play in our perception of odours and flavours with a special focus on the effect of colour. It is worth noting, however, that it is not just the colour of food that affects its palatability but also other visual features such as shape (Rolls et al. 1982) and the context in which it is presented.

1.4.1 Intersensory facilitation effects

Experimental psychologists have long been interested in the nature of crossmodal interactions between vision and gustation/olfaction (Allen and Schwartz 1940; Börnstein 1936; Gregson 1964; Hartmann 1933; London 1954; Symons 1963). The majority of this early research was designed to investigate the extent to which stimulation in one sensory modality affected the perceptual sensitivity for stimuli presented in another sensory modality, an effect termed intersensory facilitation. Most

frequently, researchers investigated the extent to which gustatory and/or olfactory cues influenced visual perception. For example, in 1936 Walter Börnstein published an experiment in which the presentation of gustatory or olfactory stimuli was reported by a majority of participants to lead to the apparent brightening or darkening of a dimly-illuminated disk presented slightly above threshold. 'Bright' taste stimuli (such as cane sugar) and 'bright' olfactory stimuli (such as citronellol 1) were reported to make the disk appear brighter, while 'dark' tastes (such as magnesium sulphate) and 'dark' smells (such as xylenol) made the disk appear darker (and in some cases even disappear). This effect was reported in 35 out of the 50 participants tested (i.e., 70% of the participants). Unfortunately, however, the explanatory validity of these results is uncertain given that the classification of a taste or smell as either bright or dark appears to have been made retrospectively (Gilbert 1941). Moreover, closer examination of the verbal reports given by participants in this study¹ suggests that the volunteers investigated in this experiment might not have been representative of the normal population and that the study itself may have been open to task demands and/or experimenter expectancy effects.

Similarly, Symons (1963) demonstrated that people's sensitivity to visual stimuli could be improved by the presentation of either olfactory (cotton wool soaked in oil of cloves placed under the nostrils) or gustatory stimuli (the periodic consumption of a sugar cube). Thresholds for detecting thin white lines presented on a

¹For example, on being presented with the 'bright' smell citronellol-1, one dark-adapted participant exclaimed: "Oh! How beautiful! *Very bright! Very bright!* It shines like the Milky Way, little dots like needle-points! . . . Now they are disappearing." (italics in original; Börnstein, 1936; p. 122). Comments such as these are more reminiscent of the accounts given by those experiencing synaesthesia, than with genuine 'normal' intersensory perceptual facilitation effects.

black background improved by approximately 6% when participants were tested in the presence of either the olfactory or gustatory stimuli as compared to performance in a baseline condition where no auxiliary stimulation was presented. A number of other researchers have also reported that accessory gustatory and/or olfactory stimuli can facilitate visual perception across a range of visual discrimination tasks (e.g., citronellol and xylol odor by Hartmann, 1933).

Although a number of theories for such intersensory facilitatory effects have been put forward (London 1954), the most likely explanation today would seem to be a generalized alerting effect, such that these accessory olfactory and gustatory stimuli (or auditory or tactile stimuli, as used in other studies) simply increased the participants' overall level of arousal (Posner 1978). This, in turn, led to a generalized facilitation of performance that just happened to be assessed by means of some sort of visual discrimination task.

Researchers have also attempted to determine whether crossmodal facilitation operates in the opposite direction as well - specifically, whether changes in ambient illumination can modify gustatory sensations. For example, participants in a study reported by Gregson (1964) were required to rate the relative intensity of pairs of sequentially presented citric acid samples (presented at concentrations of .00115, .00100, and .00085 g/ml) using a 7-point intensity scale. The two samples could either be presented at the same or different ambient illumination levels, and changes in the level of ambient lighting were shown to affect intensity judgments. Importantly, however, there were large individual differences between participants, such that while increasing the ambient illumination resulted in consistently increased intensity ratings for some participants, it had the reverse effect on others.

While such mixed results might appear at first glance to be consistent with the arousal account, given that visual accessory stimuli have been reported to be less alerting than stimuli presented to other sensory modalities (Posner et al. 1976), it is important to note that such an account would predict that the presentation of auditory accessory stimuli ought to facilitate olfactory/gustatory judgments and vice versa. However, researchers have not consistently found such an intersensory facilitation effect using auditory stimuli (McFadden et al. 1971). This apparent specificity to olfactory-visual stimulus combinations is an indication that visual-olfactory facilitation effects are indeed a 'real' phenomenon and cannot be simply attributed to a general alertness effect.

1.4.2 Visual influences on flavour perception

Here it is important to limit the discussion to studies investigating the effect of changes in the colours of foodstuffs, even though it is not only the colouring of a food item, but also its shape and the context it is presented in, which appears to influence flavour perception. For example, it has been shown that the preference for both almonds and pasta can be influenced their shape (Baker et al. 1961; Rolls et al. 1982).

The use of colorants in food to make it appear more appetizing, as well as enhance the perceived flavour, dates back many centuries. The Romans, for example, used saffron and other spices as a yellow colouring agent in various foods. Not surprisingly, the first experimental studies investigating the effect of visual attributes on flavour perception focused on coloured food items.

In one of the earliest studies to investigate the effects of colours on flavour perception, Moir (1936) demonstrated the effect of inappropriate food colouring on

the perception of a range of food products (including fruit jellies and sponge biscuits). He found that the flavour of the fruit jellies (vanilla, orange, lime and lemon) was very frequently misidentified when they were incorrectly coloured (yellow, green, amber, and red). Though short on details, he also notes that the strongest flavours were ascribed to the most highly coloured jellies. One problem about this early study is the lack of a baseline measure of people's ability to discriminate flavours in the absence of colours, so it remains unclear whether the flavours themselves were discriminable. Interestingly, Rolls and colleagues (1982) reported somewhat similar results using coloured chocolate sweets (Smarties). These candy-covered chocolates are identical but for the colours of their shell. Many of the participants in this study had a preferred colour and what's more, claimed that they could tell a difference in taste, with those sweets having their preferred colour tasting somewhat sweeter.

In another early study, Karl Duncker (1939) investigated the effect of the colours of white chocolate (which had only just been introduced into the market in North America) on flavour perception. Seven participants sequentially tasted brown and then white versions of the same chocolate, first while blindfolded and then when they were allowed to see the chocolate itself. The verbal reports of the participants suggested that that white chocolate tasted less like chocolate than the customary brown product. In particular, of the experimentally naive participants, 4 judged the white chocolate milkier than all the other samples (including the same white chocolate they had tasted while blindfolded earlier) when they could see it, while the remaining participants judged the white chocolate to have little or no chocolate taste when they could see it. However, in four subsequent experiments Duncker (1939) reports that if the participants were explicitly instructed to compare the stimuli in the various conditions, the colour effect disappeared. This suggests that participants had either

become aware to the purpose of the experiment and so were able to respond accordingly, or else were able to override the modulatory effect of visual cues when made aware of the potential discrepancy between the colours of the chocolate and its taste.

It is possible that we all have a general predisposition to attend to visual stimuli, especially in the case of food perception where we often see the food long before we taste it. This attentional bias toward the visual modality, often referred to as visual dominance (Posner et al. 1976), may help to explain why visual cues can so dramatically bias flavour perception. It may have been that the instructions in the latter part of Duncker's experiment reduced the extent to which attention was directed to vision, thus reducing the influence of visual cues. Perhaps vision will dominate our flavour perception in situations where we do not explicitly have reason to believe that we are being tricked. Under these latter conditions, people may be able to direct their attention to the gustatory and olfactory modalities and so improve the veridicality of their flavour judgments (Spence et al. 2001a; Spence et al. 2001b).

1.4.3 Dimensions of olfactory and flavour judgments modulated by colours

Taken together, these early studies suggest that visual cues can modulate olfactory and flavour perception. Colours especially appear to have a dramatic effect on our appreciation of foodstuffs, and our responses to them. However, research in this area has been controversial, with some researchers demonstrating robust effects of colours on smell and flavour perception, while others have failed to find convincing effects (see (Clydesdale 1993) for a review). Altogether, the question thus

seems no longer to be one of whether colours per se can affect flavour perception, but which attributes of smell and taste can be influenced under what circumstances. In the following section, we discuss three aspects (intensity, identity & pleasantness) of olfactory, gustatory and flavour judgments that can be influenced by colours.

1.4.3.1 Intensity

Adding colours to an odorous solution can alter the perceived intensity of the odour, an effect which has been reported in numerous studies. Interestingly, people are far more likely to report that an odourless stimulus smells if it is coloured than if it is colourless (Engen 1972), suggesting that they expect a coloured substance to have a smell. Similarly, a study by Zellner and Kautz (1990) found that adding colour (e.g. red) to an odorous liquid (e.g. strawberry) enhances the perceived intensity of its odour. The authors also reported that some participants in their study simply refused to believe that coloured and uncoloured solutions of equal odour concentrations were actually equally strong! Often, the perceived increase in the intensity of the odour depends on the intensity of the colour that is added. For instance, Zellner and Whitten (1999) found that people's rating of the intensity of the odour of a mint-flavoured drink increased monotonically as they increased the intensity of the green colouring. In a similar study, using artificial odours without any associations to foods or other objects, it was found that increasingly stronger smelling odours were associated with darker visual stimuli of the same hue (Kemp and Gilbert 1997).

The effect of colours on taste has proven to be somewhat weaker than to olfaction, and research has produced mixed results. For example, Pangborn (1960) evaluated the effect that colours (colourless, red, green or yellow) can have on

intensity judgments of sucrose solutions either in water or in fruit nectars. Subjects had to indicate the sweeter solution in a paired comparison task using concentrations of about 5%. No significant differences in perceived intensity were found for the aqueous solutions. If fruit flavoured solutions were used, subjects showed a slight (but not statistically significant) tendency to ascribe greater sweetness and greater flavour to apricot and cherry flavouring if they were coloured orange and red respectively, especially when the sucrose differences were very small. This would suggest that appropriate colours-flavour combinations such as cherry-red might produce enhancement effects. Interestingly, when pear nectar was used as the base (again with 5% sucrose), green-coloured samples were judged less sweet than nectar containing the other colours, possibly because of an association between green and unripe fruit. However, in a later study Pangborn and Hansen (1963a) evaluated the response to sourness and sweetness levels (5.0% vs. 5.2%) in coloured (red, yellow, green and blue) and uncoloured pear nectar and found that correct discrimination responses were more frequent in uncoloured than coloured nectar pairs. This implies that the addition of colours has a deleterious effect on sucrose discrimination. Unfortunately, they did not find a tendency for people to judge green-coloured nectar as less sweet or uncoloured nectar as sweeter, contrary to previous results.

On the other hand, a later study (Maga 1974) found a colouring effect on taste thresholds for solutions of 4 of the basic tastes (sweet, sour, bitter and salty, created by sucrose, sodium chloride, citric acid and caffeine respectively) that were either colourless or coloured red, green or yellow. The results clearly demonstrated that the addition of colours affected people's ability to distinguish threshold levels for the basic tastes. In most cases, coloured solutions had to be more concentrated before people could identify the specific taste, especially for sour and bitter tastes.

Importantly, however, the degree of insensitivity varied according to the colours. More specifically, adding green (but not the other colours) to a sweet solution increased its perceived sweetness (i.e., lowered threshold), while yellow and red increased threshold. Interestingly enough, taste sensitivity to salt (sodium chloride) showed no significant changes with any of the colours. The discrepancy in findings between the studies of Pangborn and Maga are most probably a result of the concentrations of sugar solutions used (threshold in Maga's study and 5% in Pangborn's). It is a well known phenomenon that multisensory enhancement shows the largest effects if stimuli are presented near threshold level (Stanford et al. 2005).

The implications of intensity enhancement by colours could be quite considerable: if one could use food colourings to achieve such colour-induced intensity augmentation, this would provide a much healthier means of sweetening drinks than actually adding sugar and could also decrease the amount of salt in savoury foodstuff. This technique could be particularly important for those on low-sugar or low-sodium intake diets. Additionally, colour-induced taste enhancement might also be very important for the elderly, who find it much harder to taste sugar in food than younger people, largely due to diminished olfactory capabilities (Cain et al. 1990; Clydesdale 1994; Stevens et al. 1991).

1.4.3.2 *Identity*

Colours cues can not only change the perceived intensity of the flavour of food and drink, but the actual flavour itself. This effect is probably a consequence of our poor performance in identifying odours in the absence of visual cues (Desor and Beauchamp 1974). Under blindfolded conditions, subjects can usually only correctly

name one third of the presented odours, even if the stimuli used are highly familiar (e.g. smell of leather).

In one classic study, DuBose and colleagues (1980) demonstrated that while the majority of people perceived a cherry-flavoured soft drink as tasting of cherry when it was coloured cherry-red, many thought it tasted of lime when coloured green, and nearly 20% of people thought it tasted of orange when coloured orange. Similar effects have also been reported for flavour identification. Richard Hall (1958) reported an experiment conducted on one sensory panel at McCormick & Company. They used sorbets of different flavours (lemon, lime, orange, grape, pineapple, and almond), each presented in different trials in each one of three colours: the commonly associated (or natural; congruent) colours, an inappropriate (or incongruent) colour, and white or uncoloured. Participants were generally successful in identifying the flavours when they were associated with their 'normal' colours (e.g., 75% correct identification when the colours were congruent; e.g., green for lime, yellow for pineapple), but made many identification errors when the colour was lacking (performance dropped to 46% correct). When the sorbet was incongruently coloured only a few people were able to name the flavour correctly (performance dropped to 20% correct), and most named a flavour normally associated with the colours presented. Interestingly, with the one exception (that of the almond-flavoured sorbet), congruently coloured sorbets were also rated highest in flavour acceptability (i.e., on a hedonic scale) while incongruently coloured sorbets generally received the lowest ratings (see the next section for a discussion of visual influences on odour pleasantness).

In a comparable study, Anton Teerling (1992) investigated the effect of colours on flavour identification of wine gums which were either coloured according

to the actual taste (e.g., red for strawberry), colourless, or else incongruently coloured (e.g., colouring a peach flavoured wine gum purple). Participants were given the list of all the possible flavours they would encounter. In this study, taking the colours away from wine gums was found to have no effect on flavour identification performance, although once again performance fell dramatically (by 46%) when people tried to identify the incongruently coloured samples.

In summary, there is clear evidence that the colours of both foods (Hall 1958; Moir 1936; Teerling 1992) and drinks (DuBose et al. 1980) can play an important role in determining the perceived identity of foodstuffs with colours sometimes facilitating, but always interfering with identification when inappropriate. Although hard to quantify, the weight of the empirical evidence published to date seems to support the view that the dominance of colour cues in determining flavour identity increases as the quality of the gustatory and olfactory cues declines. It is important to note, however, that in the majority of these studies, it appears that participants were never informed that the colours might be misleading. As such, the results may simply reflect the fact that participants did not properly perceive the odour that they reported. Instead, since they may have found it difficult to discriminate the odour, they could have simply decided to respond on the basis of the more easily discriminable colours. An important methodological improvement for such studies would involve explicit instruction that odour/taste and colours are varying independently.

1.4.3.3 *Pleasantness*

The pleasure we derive from consuming food and drink can also be highly affected by how it looks, with colours again being a major component of the overall

appearance (Johnson and Clydesdale 1982; Teerling 1992). People prefer odours, such as the smell of a fruit, if they deem the colours to be appropriate (Christensen 1985; Zellner et al. 1991), and also like food and drink more if it tastes as they expect (Cardello and Sawyer 1992). For example, Zellner and colleagues (1991) presented their participants with fruit odours in solutions that were coloured appropriately (e.g. cherry –red), inappropriately (e.g. cherry – green) or under blindfolded conditions and found that the participants rated the appropriately coloured solutions as the most pleasant. However, this effect may have been due to the increase in identification accuracy of the appropriately coloured solutions, since across all three conditions, correctly identified odours were liked more than odours that were not correctly identified. Therefore, the ability to identify an odour may influence the affective response.

On the other hand a study conducted by Christensen (1985) has shown that people rate everyday foods - such as margarine, orange juice, bacon, and cheese - as being of better quality and having a stronger aroma when coloured normally than when they were either presented colourless, or else coloured inappropriately. Since their participants were able to correctly identify the foods in all conditions, this provides convincing evidence that colouring per se may influence liking for foods.

There are, however, even more dramatic examples of the effect of colouring on the perceived pleasantness of food. In one extreme study reported by Wheatley (1973), a group of people were gathered around a table and given a meal of steak, chips and peas. The people initially enjoyed eating the food that appeared normal under special lighting. However, after they had consumed some of the food, the lighting was returned to normal, which resulted in people suddenly realizing that the steak they were eating was actually blue, the chips green, and the peas red! According

to Wheatley, on seeing the foods' real colours, almost all of the people were violently sick. Although Wheatley gives scant details regarding the experimental details of this particular study, it nevertheless suggests just how powerful visual cues are on our affective perception of food.

In a similar study, Rozin and Fallon (1987) showed that visual cues can be used to set up such powerful contextual associations that people will simply refuse to consume food. For example, nurses in a children's hospital were inappropriately consuming glasses of juice meant for the children. To rectify this situation, the juice was then served in new urine-collection bottles. This change in the drinking vessel resulted in the nurses no longer drinking the juice, even though there was no possibility of a physical trace of urine in the bottles. Similarly, people are reluctant to eat a favourite food if it was in contact with an item that looks disgusting. For example, about half the subjects surveyed by Rozin and colleagues reported a substantial drop in the acceptability of their favourite soup after it has been stirred by a brand-new fly swatter or a brand-new comb (Rozin et al. 1984).

Here it is important to realize that smell and taste cues usually dominate our perception of the pleasantness of food, especially since once the food is ingested, all visual cues are eliminated. If, for example, people came across food that looked extremely appetising, but which smelled or tasted off, they would simply refuse to eat it, no matter how attractive it appeared. This demonstrates that smell and taste cues can dominate our judgments of the overall palatability of food. Consequently, it is clear that no one sense alone always dominates our perception of food.

In sum, research on visual-olfactory interactions suggests that food scientists may soon be able to increase the enjoyment people derive from consuming food and drink by carefully selecting the colours that best complement and enhance the taste

and smell of particular products (Gilbert et al. 1996; Kemp and Gilbert 1997).

Furthermore, the careful use of enhanced colours cues for certain groups of people, such as the elderly or those on low-sugar intake diets, may also provide an effective means of enhancing the flavour of food.

1.4.4 Neural mechanisms for multisensory visual-olfactory interactions

As detailed above, scientists (mainly in the food and fragrance industry) have provided ample behavioural evidence that visual cues, and in particular colours, may influence the basic dimensions of olfactory perception, namely odour identification, liking and intensity. Unfortunately, comparatively little is known about the underlying neuronal mechanisms responsible for these effects. In the following section, the principles of multisensory integration in the context of fMRI are discussed, followed by a review of the current neurophysiological evidence for visual-olfactory interactions.

1.4.4.1 *fMRI principles of multisensory integration*

To date numerous human brain imaging studies have sought to identify the brain regions involved in multisensory processing (see (Calvert 2001) for a review). Even though multisensory interactions have been mainly studied for other sensory combinations (e.g. audio-visual) than the chemical senses, some generic rules that have emerged seem also to apply to visual-olfactory combinations. The design of many of these imaging studies has been influenced by electrophysiological studies in

animal models which have attempted to characterise the responses of individual multisensory neurons to the presentation of cues from different sensory modalities (Meredith and Stein 1983; Stein 1998; Wallace et al. 1992). These studies have been carried out primarily in the superior colliculus (SC) of cats and monkeys. The SC is an attractive model to study multisensory integration, because of its high incidence of multisensory neurons, its involvement in overt orientation behaviours such as saccades. However, the same mechanisms found in SC neurones have since been demonstrated to exist for cortical multisensory neurones as well (Avillac et al. 2007). Two characteristic responses in multisensory neurones have been identified in both SC and cortex:

Firstly, response enhancement, in which the multisensory response exceeds either of the unisensory responses and secondly, response depression, whereby the multisensory response is less than the best responding of the two unisensory cues. In the case of audio-visual stimuli, such crossmodal enhancements and depressions of activity have been found to be largely dependent on the spatial and temporal relationships of the combined stimuli. For example, response enhancements occur when stimuli that are spatially and temporally coincident are combined, whereas response depression can be observed when stimuli from different modalities do not share spatial or temporal correspondence (Wallace et al. 2004a). The observation of either response enhancements or response depression in a single neurone is taken as evidence that sensory cues originating from the different modalities are integrated within this neurone.

Based on these principles derived from electrophysiological recordings in animals, most human fMRI studies have been designed to test the hypothesis that multisensory brain regions will exhibit enhanced responses to spatially and temporally

congruent stimuli and depressed responses to incongruent stimuli (Bushara et al. 2001; Calvert 2001; Calvert et al. 2004; Macaluso and Driver 2005). The behavioural consequences in humans of such processes of multisensory integration can then be typically observed in the form of shortened detection time and increased accuracy for congruent stimulus combinations and a decrease in such psychophysical performance for incongruent combinations.

At this point it is important to note that the signal being measured in functional imaging studies is derived from a large population of neurones, a fact that may make comparisons to the behaviour of single neurons somewhat problematic (Calvert & Thesen, 2004). This is particularly true in view of the fact that the neuronal population in a given region of cortex at the resolution of fMRI (typically in the region of 50mm^3) is likely to be heterogeneous. It is therefore likely that unisensory neurones (i.e. neurones that exclusively process information from one sensory modality) co-exist with multisensory ones. Electrophysiological recordings in the primate OFC have indeed revealed that neurones with diverse response properties (e.g. unimodal and multimodal neurones responding to taste, olfaction or vision) are located in close proximity to each other (Rolls and Baylis 1994; Rolls et al. 1996a). Accordingly, the response to multisensory stimulation in such a large neuronal population is ambiguous, since it could originate exclusively from multisensory neurones, from the co-activation of both sets of unisensory neurones, or some combination of these two. Therefore, the finding of enhanced activation in response to multisensory stimulation, even at the level of a single voxel, does not provide unequivocal proof of convergence, since an enhanced response to two unisensory stimuli might simply reflect the activation of two independent populations of neurones and not the convergence of these inputs onto multisensory neurones.

To address these methodological issues, more rigorous methods than simple response enhancement or depression have been suggested for assessing putative sites of multisensory processing. In their excellent reviews of these issues, Calvert (2001) and Laurienti and colleagues (2005) noted that the following criteria have been used in previous fMRI investigations as evidence for multisensory integration in a given area of the human brain:

- 1. The area responds to stimulation in two different sensory modalities.*
- 2. The area responds more strongly to the multisensory stimulus than to either of the unisensory stimuli.*
- 3. The area responds to multisensory stimulation, but not to either unisensory stimulus.*
- 4. The area responds more strongly to congruent multisensory stimulation than to incongruent stimulation.*

However, these criteria still do not constitute unequivocal proof that a given brain region is actually involved in the integration of inputs from two sensory systems. As mentioned above, any brain region responding to both unisensory modalities could simply be comprised of two separate populations of unisensory neurons located within the same voxel (criterion 1). Likewise, the same area would show a greater signal under multisensory conditions than under unisensory conditions (criterion 2) because this signal would simply be the sum of the signal generated by the unisensory populations. Satisfying the third criterion could purely be the result of a statistical thresholding phenomenon, where the signal is strong enough to be detected only under multisensory conditions. Lastly, using criterion 4 could lead to

brain regions being described as multisensory even if these could simply be responding to stimulus congruence independently from the sensory modality employed. Consequently, each of the criteria described above are highly susceptible to false positive errors, thus leading to regions being wrongly identified as multisensory when they are exclusively comprised of unisensory neurones.

In order to rule out such potential false positive errors in neuroimaging data, the metric of superadditivity has been suggested as the strongest evidence for multisensory integration (Calvert 2001; Laurienti et al. 2005). Superadditivity means that the response to the multisensory stimulation must be greater than the sum of the unisensory responses (Figure 1.4). Since superadditive signals in a given brain region may not be generated by the summed activity of unisensory neurones alone (additivity), the observation of superadditivity is seen as strong evidence for integration of multisensory signals by multisensory neurones.

It is important to note, however, that even though superadditive responses can be observed during electrophysiological recordings from multisensory neurones (Meredith and Stein 1983; Stein 1998; Wallace et al. 1992), many multisensory neurones behave in an additive or even subadditive fashion when stimulated by multiple sensory modalities (Perrault et al. 2005). Additionally, in their theoretical calculation (based on known spiking rates for both unimodal and multisensory stimulation) of the BOLD responses elicited within a region of the superior colliculus containing only multisensory neurones, Laurienti et al. (2005) predicted that the multisensory response would not exceed the sum of the unisensory responses, but would instead be slightly subadditive.

With the emerging evidence that even at the level of single neurones, superadditive effects may be restricted to stimuli presented near threshold level

(Perrault et al. 2005), the metric of superadditivity may be considered overly conservative for fMRI investigations. Consequently, the experimental designs and analytic strategies used for multisensory fMRI studies have been increasingly incorporating parametric paradigms (where one or more stimulus aspects are varied parametrically) rather than the superadditive metric.

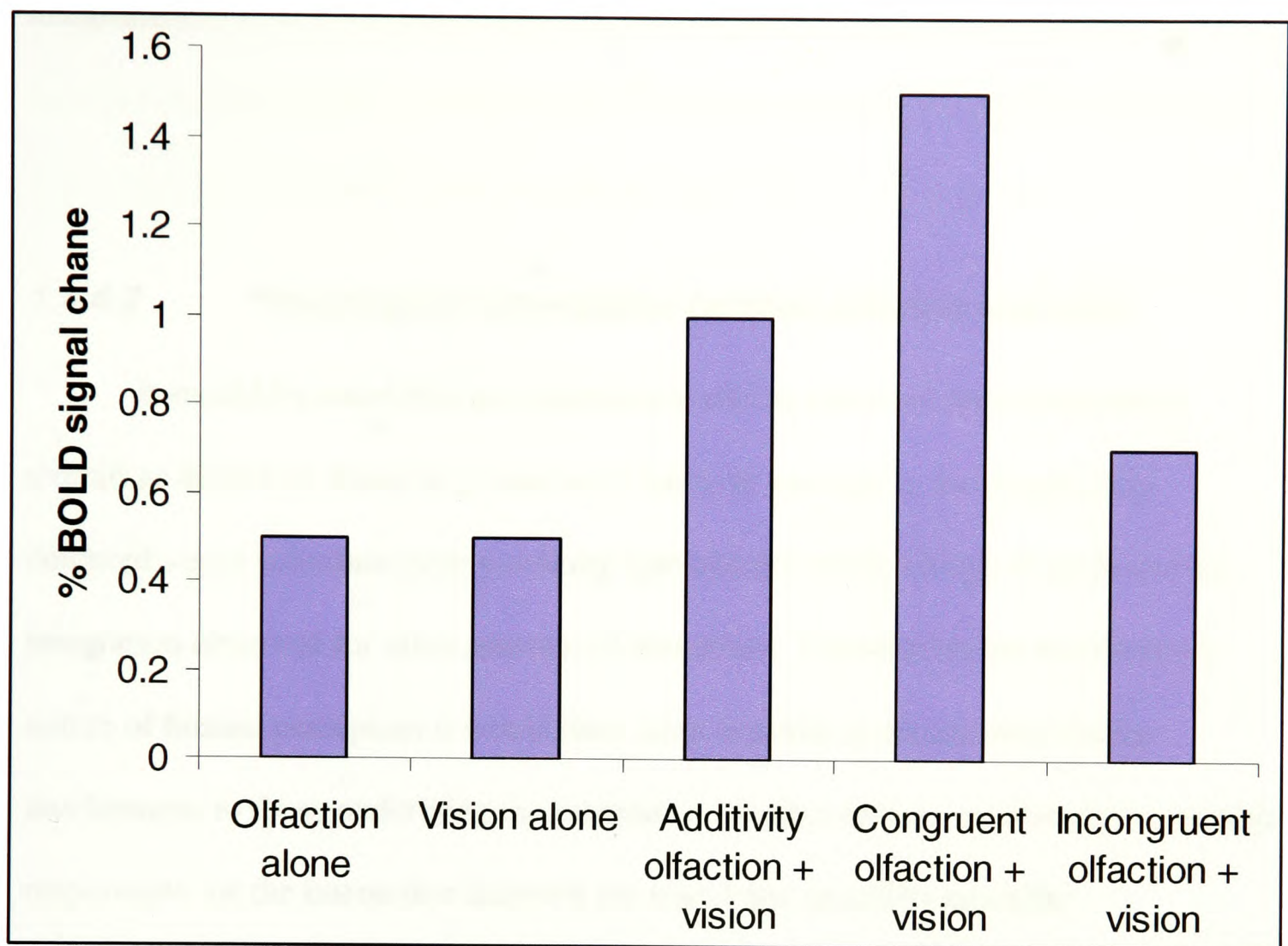


Figure 1.4: Effects of super- and subadditivity.

The graph shows a hypothetical outcome of a multisensory fMRI experiment using visual and olfactory stimuli either in isolation or in congruent and incongruent bimodal combinations. The percentage BOLD signal change in response to congruent olfactory-visual stimuli exceeds those predicted by the sum of the two unimodal ones (superadditivity). Conversely, incongruent olfactory-visual stimuli elicit less of a response than the sum of the unimodal ones (subadditivity), therefore displaying a response depression.

To conclude, it may be said that even though several different criteria have been put forward as evidence for multisensory integration in neuroimaging studies, some of these may be not strong enough to rule out the possibility of false positives. Whilst the criterion of superadditivity is undoubtedly the best metric to show multisensory brain regions, it may prove to be overly conservative in some cases

(generating false negatives) and therefore failure to demonstrate superadditive effects in fMRI studies should not necessarily be seen as evidence against multisensory integration.

1.4.4.2 *Physiological convergence between olfaction and vision*

It should be noted that the behavioural effects observed for colour-odour stimuli as described above (e.g. increased intensity perception for congruently coloured odour solutions) bear a striking resemblance to the effects of multisensory integration observed for other sensory combinations. Considering the multisensory nature of human perception it would then seem sensible to assume that similar mechanisms to those underlying the integration of other sensory combinations are also responsible for the interaction between the visual and olfactory modality.

The most likely candidate for such interactions is the OFC, since it receives inputs from both the visual and the olfactory system. There are direct projections from the primary olfactory cortex (piriform cortex) to area 13a of the posterior OFC, which in turn projects to the medial parts (area 11) of the OFC (Barbas 1993; Carmichael et al. 1994) (see Figure 1.5). Additionally, projections from the visual system reach the OFC via the inferior temporal cortex and the superior temporal sulcus (Barbas 1988; Morecraft et al. 1992). Similarly, the OFC appears to be crucially involved in flavour perception (i.e. the integration of olfactory and gustatory inputs) and the caudolateral part of the OFC receives direct inputs from the primary taste cortex located in the insula cortex and adjoining frontal operculum (Rolls et al. 1990; Yaxley et al. 1990).

Comparable inputs from the visual, gustatory and olfactory system are also received by the amygdala (Price 2003) which in turn projects to the OFC (Porrino et

al. 1981). Particularly the basal nuclei of the amygdala contain the highest concentrations of neurons projecting to the OFC. The OFC in turn projects to the several amygdaloid nuclei, namely the basal, accessory basal, lateral, central, paralamina and anterior cortical nuclei as well as to the periamygdaloid cortex and the amygdalo-hippocampal area (Cavada et al. 2000).

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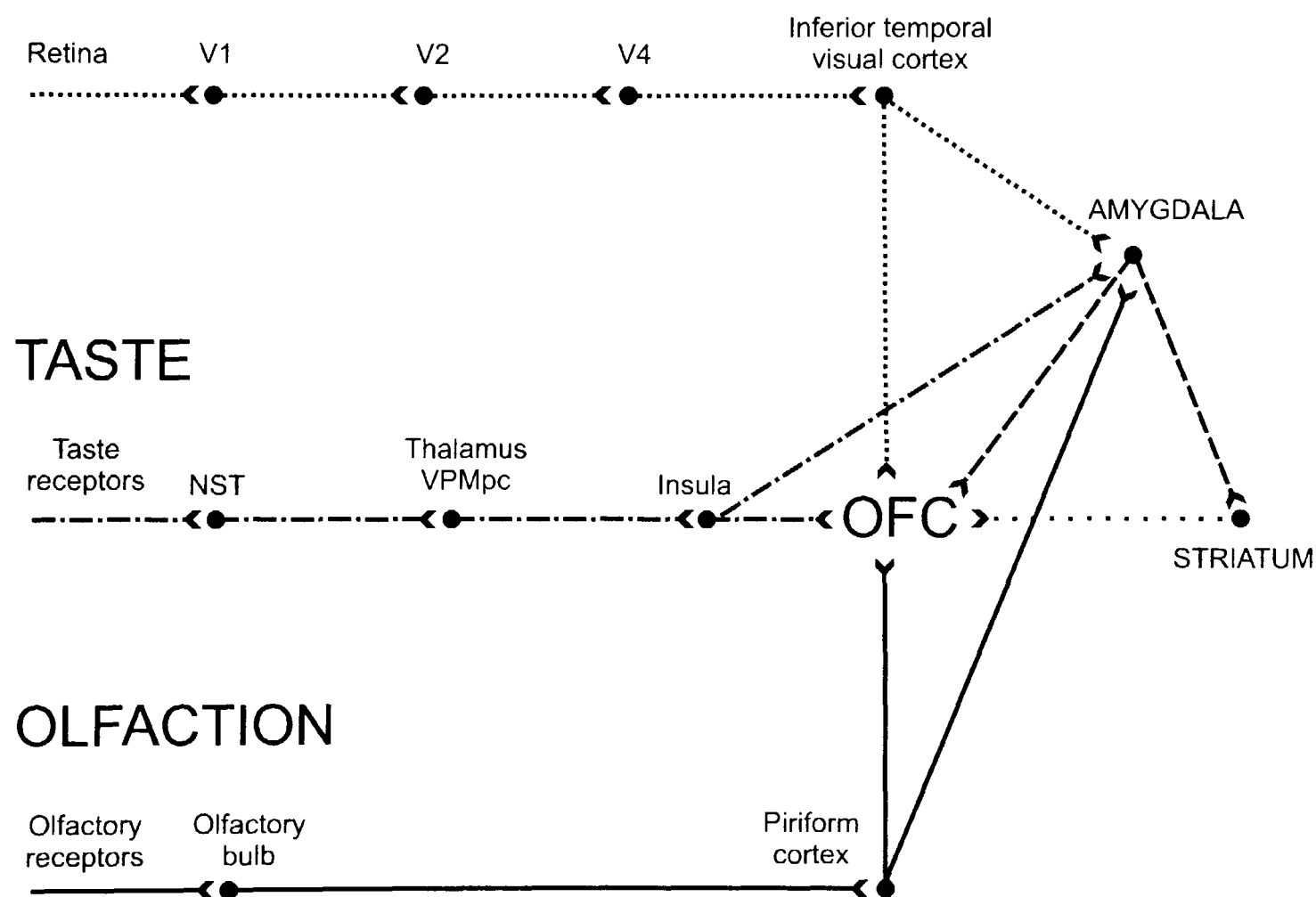


Figure 1.5: Sensory inputs to the OFC.

The schematic diagram illustrates the convergence of visual, gustatory and olfactory inputs in the OFC. V1, V2, V4 visual cortical areas; NST, nucleus of the solitary tract; VPMpc ventral-posteromedial thalamic nucleus (after Rolls, 2004).

Most of our current knowledge about multisensory integration involving olfactory cues at the neuronal level has been derived from electrophysiological recordings in primates, mainly conducted by Edmund Rolls and colleagues (Critchley and Rolls 1996a; 1996b; Rolls 2004b; Rolls and Baylis 1994; Rolls et al. 1998; Rolls et al. 1996a; Rolls et al. 1996b). These studies have demonstrated that it is particularly within the OFC that multisensory interactions between olfactory and visual inputs occur. For example, in their pioneering study, Rolls and Baylis (1994) demonstrated that many of the neurones within the caudal OFC that responded to visual stimuli also responded to olfactory and taste stimulation. Since such multimodal neurones were found in very close proximity to unimodal neurones (responding exclusively to vision, olfaction or gustation), the authors concluded that the OFC represents the first cortical area of convergence for these three modalities in primates, including humans. Additionally, many of the OFC neurones respond to the sight of food, but only when hunger is present (Critchley and Rolls 1996a). Therefore, the OFC seems to provide a neural circuit that can alter the responses to a visual stimulus dependent on the reinforcement value associated with it and hence a mechanism for the sight of food or drink to influence its flavour.

Investigations of vision and olfaction in a multisensory context using neuroimaging techniques have so far been relatively rare. In the first published studies that the author is aware of, Grigor et al. (1995; 1999) used event-related potentials (ERPs) to investigate the effect of matching versus mismatching smell–picture combinations with olfaction as a prime for both food-related and non-food-related stimuli. In both studies, the olfactory prime was delivered for 4s prior to the presentation of either a matching or a mismatching visual stimulus (e.g. the smell of a rose and the picture of a rose vs. the picture of a cricket bat). The results in both cases

demonstrated a difference in the negative ERP waveform 400ms after the onset of the visual stimulus (N400) when the odour and the picture did not match compared to the matching condition. Even though this effect was strongest over frontal electrodes, the authors did not specify whether the source of activation could originate from the orbitofrontal cortex. Additionally, since the two stimuli were presented consecutively and not simultaneously it seems likely that the observed effect could have been caused by stimulus incongruency rather than cross-modal stimulus integration.

The first fMRI investigation using congruent and incongruent odour-picture pairs as well as unimodal stimulation was conducted recently (Gottfried and Dolan 2003). Participants in this study were required to detect the presence or absence of odours. The behavioural results demonstrated that the participants detected odours faster when both visual and olfactory stimuli were congruent, compared to incongruent combinations or odours in the absence of visual stimuli. Furthermore, these perceptual changes were associated with enhanced neural activity in both the anterior hippocampus and the rostromedial OFC (a more detailed discussion of the results of this study can be found in Chapters 7&8).

A somewhat separate question that arises in the context of these visual–olfactory interactions is how these cross-modal associations are acquired. A recent event-related fMRI experiment investigated the neuronal mechanisms responsible for olfactory learning with a classical conditioning paradigm (Gottfried et al. 2002c). In this study three pictures of ‘neutral’ faces were repeatedly paired with any one of a pleasant, a neutral, or an unpleasant smell under a 50% reinforcement schedule while a fourth ‘neutral’ face remained unpaired (control condition). The results of this study demonstrated that the face paired with a pleasant odour evoked significantly stronger activation in medial orbitofrontal regions, the ventral striatum, and the amygdala

compared to the neutral (unpaired) face. In contrast, the face paired with the unpleasant odour resulted in peaks of activity in the left lateral and right medial orbital gyri. Even though some brain regions appeared to be specifically engaged in olfactory learning depending on the valence (pleasant vs. unpleasant) of the odours (such as the ventral striatum and the amygdala), widespread regions of the rostral and caudal OFC activated independently of odour valence. It is thus mainly the OFC that plays a critical role in the formation of associations between odours and visual stimuli. In sum, the neurophysiological evidence collected to date indicates that the behavioural effects observed in psychological studies have a neuronal basis mainly within the human OFC and cannot simply be attributed to experimenter effects, demand characteristics, or biases.

1.4.5 Summary and conclusions

As has been discussed, there is ample behavioural evidence that visual inputs, particularly colours, can influence the perceived intensity, pleasantness or identity of an odour. Both anatomical and physiological studies in human and non-human primates, indicate that the main brain region involved in the integration of olfactory cues and other sensory modalities is the orbitofrontal cortex. It is therefore feasible to hypothesise that it is particularly within the human OFC that olfactory information is synthesised with colour information, and is responsible for generating these behavioural interaction effects. Additionally, olfactory structures within the medial temporal lobe - in particular the amygdala, are recipients of visual and olfactory inputs, are reciprocally connected to the OFC, and therefore may be involved in the multisensory integration of both sensory streams. The primary purpose of this thesis is to investigate the involvement of these brain regions in the multisensory integration of vision and olfaction using fMRI (see Chapters 7&8).

2 Methods

Functional magnetic resonance imaging (fMRI) is a powerful, non-invasive brain imaging technique that permits the investigation of human brain function. All experiments presented in this thesis use this technique to investigate the neural basis of the human olfactory system with very high spatial resolution. This chapter describes the basic physical and physiological principles of fMRI as well the statistical analysis of the data. Additionally, the stimulus delivery systems used in the subsequent investigations are described together with a pilot study investigating the effects of respiration on brain activation.

2.1 INTRODUCTION

The major aim of this thesis is the investigation of how olfactory inputs are integrated with those from the visual modality in the human brain. Two major methodological challenges present themselves to any researcher wishing to investigate human olfaction with fMRI: image acquisition of sufficient quality in olfactory areas and the presentation of odorants.

Firstly, the olfactory regions in the orbitofrontal cortex (OFC) reside in close proximity to the air-filled nasal sinuses. This causes both geometric distortions and signal drop-out and specialised imaging methods may have to be employed in order to acquire images of sufficient quality.

Secondly, olfactory stimuli may be presented either ortho- or retronasally, i.e. either inhaled via the nostrils or reaching the olfactory epithelium via the mouth and through the posterior nares of the nasopharynx (Cerf-Ducastel and Murphy 2001; Pierce and Halpern 1996) in which case they are usually dissolved in a liquid. To date, there exists only one commercially available apparatus for odour delivery, which is suitable for the MRI environment and allows for a precisely timed stimulus presentation. This olfactometer was originally developed by Kobal (1985) together with the company Burghard (Wedel, Germany) specifically for EEG studies of olfaction and therefore has a high temporal precision with an odour rise-time of 20ms. Because of the high cost of this device, a device suitable for the fMRI environment had to be developed specifically for the work presented in this thesis. Both of the issues mentioned above are discussed in the following section.

2.2 FUNCTIONAL MAGNETIC RESONANCE IMAGING

The technique of functional Magnetic Resonance Imaging (fMRI) was developed after the discovery that blood oxygenation level dependent (BOLD) contrast can be used as a marker for brain activity in the early 1990's (Ogawa et al. 1990). Since its discovery, fMRI has become a powerful tool to study brain function in humans at high spatial resolution and without the use of radioactive contrast agents. The following section provides an overview of fMRI, from its physical principles to the generation of images based on BOLD contrast and the statistical analysis of the data to reveal activation maps of human brain function.

2.2.1 Basic physical principles of MR imaging

2.2.1.1 *Nuclear moment and spin excitation*

A basic property of atomic nuclei consisting of protons and neutrons is that they possess an angular momentum, known as 'spin' (as a convenient analogy, the nucleus can be imagined as a spinning top at a certain angular frequency). The nuclear spin is determined by the number of protons and neutrons in the nucleus. As a result of the fact that the nuclei are positively charged and possess a spin, they also generate a nuclear magnetic moment μ (Figure 2.1). Only nuclei with an uneven atomic mass or an uneven charge have a net nuclear magnetic moment, limiting the number of elements suitable for MR imaging. For the purpose of MR it is the net nuclear magnetic moment (M) of all nuclei in a sample of tissue that is of interest. The nuclei imaged during MRI experiments are those contained within hydrogen atoms (H_1) with

a quantum spin number $I = 1/2$, which have the advantage of both a very strong nuclear moment and of a natural abundance in human brain tissue.

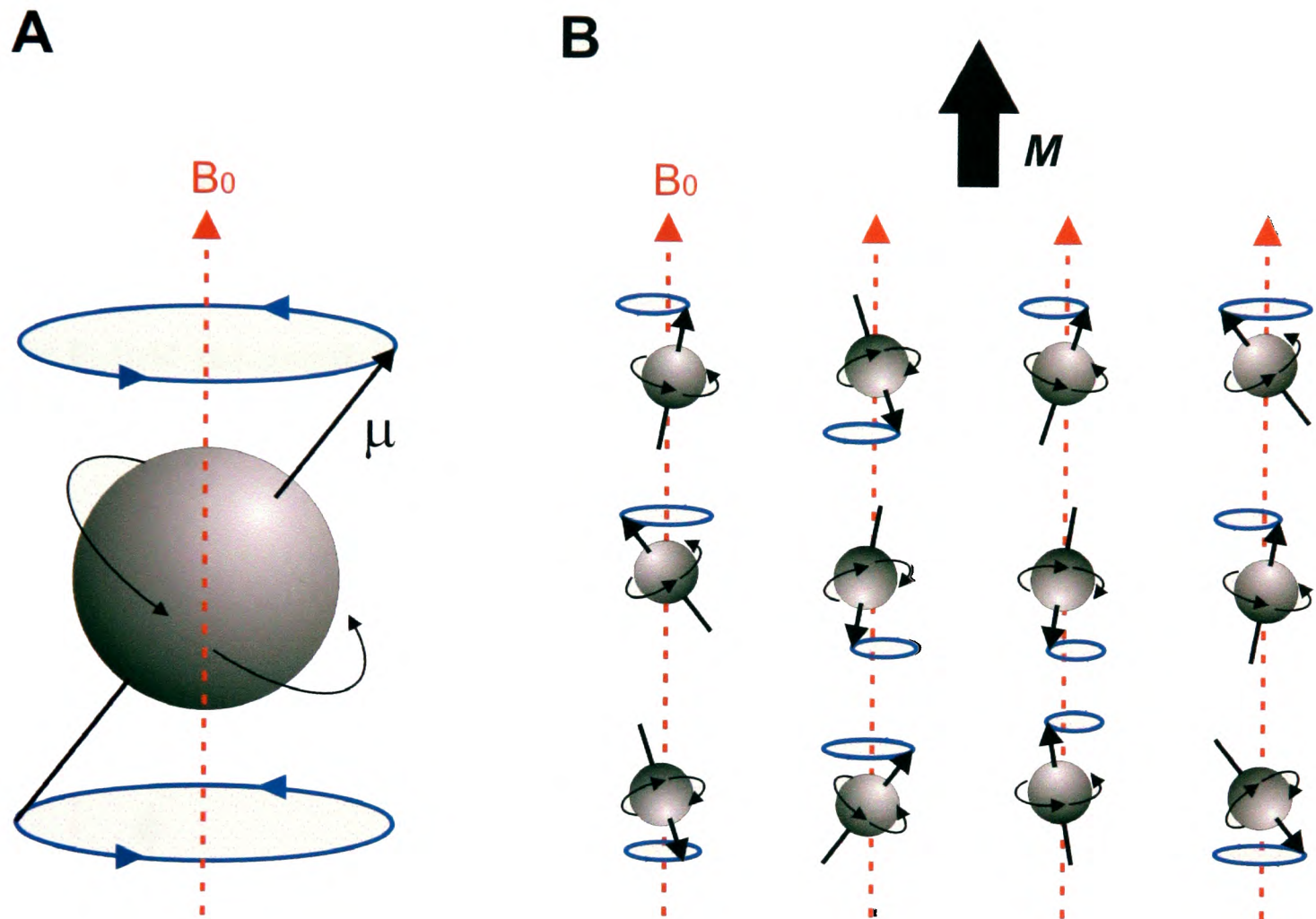


Figure 2.1: Hydrogen nuclei in a magnetic field.

(A) A hydrogen nucleus precessing at the Larmor frequency around the axis of the static B_0 field (red dashed line), thereby creating a nuclear magnetic moment μ . **(B)** The slight preferential alignment of the nuclei with the B_0 field gives rise to the net magnetisation M .

Placed in a magnetic field (B_0), an atomic nucleus can only possess certain discrete energy levels, determined by its spin number. These levels are calculated by $(2I + 1)$, which for hydrogen results in two discrete energy levels. These two levels may be imagined as two possible orientations of the hydrogen in the magnetic field: a lower energy state in parallel alignment with the field and a higher energy state in

anti-parallel alignment with the field. A sample of hydrogen atoms would naturally orient itself towards its low energy state when placed in a static magnetic field B_0 , causing a net magnetisation vector M in the direction of the B_0 field. However, at the physiological temperature of approximately 36°C the hydrogen nuclei are subject to considerable thermal agitation, which dominates the alignment of the energy states and hence the size of the net magnetisation M . Nevertheless, a very small percentage of the nuclei (0.001% for an external field of 1.5 Tesla) will align preferentially with the B_0 field and contribute to the generation of the net magnetisation vector M .

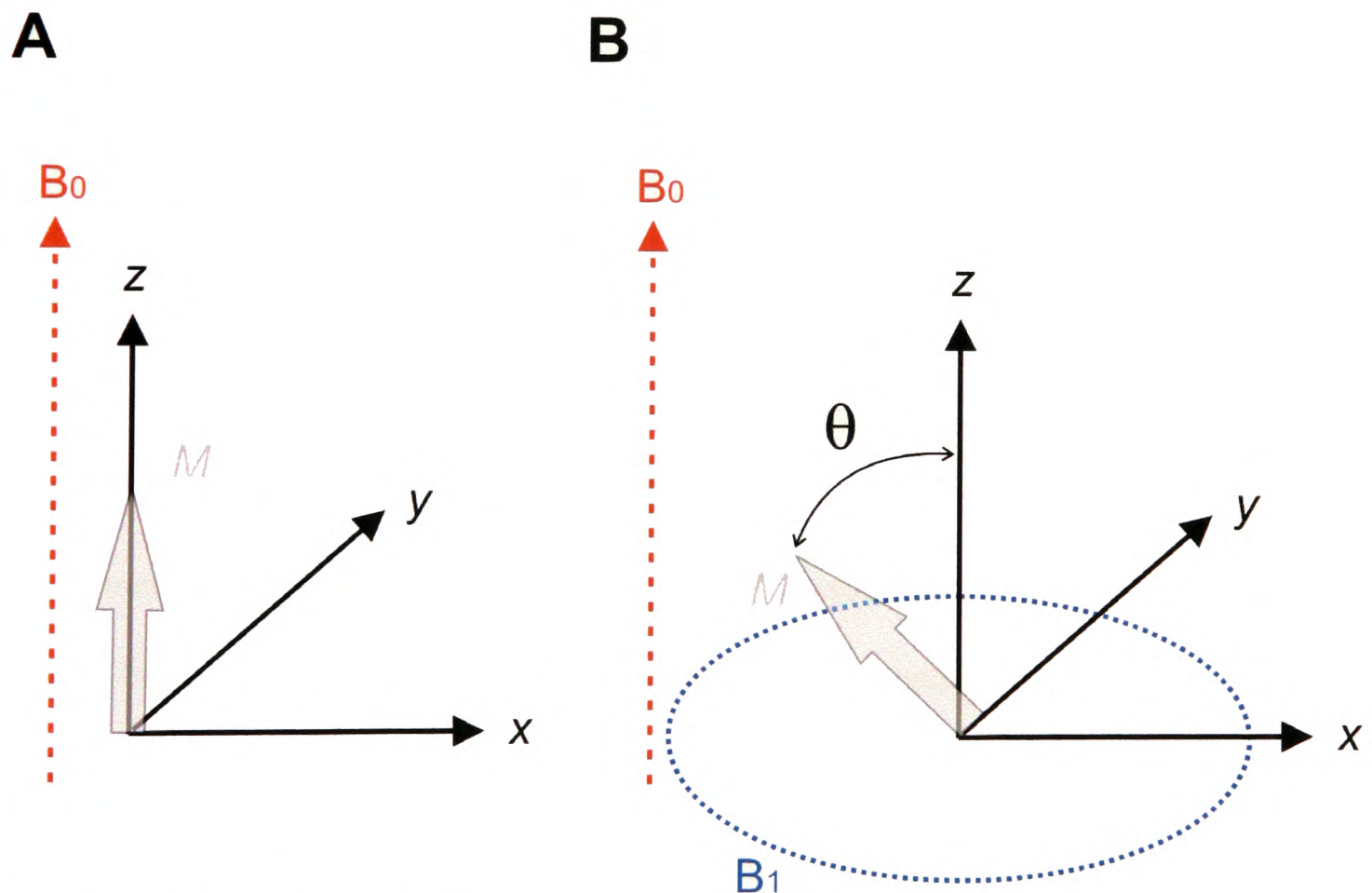


Figure 2.2: Excitation pulse.

A In the static B_0 field, the net magnetisation M is parallel to B_0 . **B** After application of a B_1 field at the Larmor frequency (excitation pulse), the magnetisation vector is tilted by an angle θ towards the transverse plane.

A fundamental property of the sample in the magnetic field is that it can be excited by the application of an additional radiofrequency magnetic field (B_1) at its resonance frequency. This excitation causes a transition of the nuclei from the low-energy state to their high-energy state, which in turn affects the net magnetisation vector M . The specific resonance frequency that needs to be applied is called the Larmor frequency (ν) and depends on the external magnetic field (B_0) and the gyromagnetic ratio (γ) of the nuclei in the sample. This relationship is described by the Larmor equation $\nu = \gamma B_0$. Since hydrogen atoms possess a gyromagnetic ratio of 42.577 MHz/T, the resonance frequency in a 3 Tesla magnet is at approximately 128 MHz. The application of the B_1 field at the Larmor frequency causes the magnetisation vector M to be tilted away from alignment with B_0 (Figure 2.2). This flip angle (θ) at which M is tilted away from B_0 is dependent on both the amplitude and duration of the applied pulse. Typically, the MR excitation pulse will be applied long enough to tilt the net magnetisation vector M by $\theta = 90^\circ$, so that it lies perpendicular to the B_0 field in the transverse plane. After the excitation pulse has ended, M will precess back to alignment with the B_0 field, in a process called relaxation. During the relaxation process, both longitudinal (M_z) and transverse (M_{xy}) components of the net magnetisation vector M return to their equilibrium values in an exponential fashion and this precession of M can be measured by a receiver coil and is called free induction decay (FID).

2.2.1.2 *Spin relaxation*

It is important to note that the relaxation processes influencing the transverse and longitudinal components are independent, causing both to give rise to different MR contrasts (Figure 2.3). Essentially, the transverse magnetisation can disappear long before the longitudinal magnetisation is restored. The time it takes for the longitudinal magnetisation to return to 63% of its original strength (spin-lattice relaxation) is characterised by a value T_1 , which varies according to the type of tissue and the strength of the external magnetic field (e.g., 1331ms for grey matter, 832ms for white matter and > 4000ms for cerebrospinal fluid at 3 Tesla) (Wansapura et al. 1999). Images acquired with T1 weighted contrast are typically used for high resolution anatomical images.

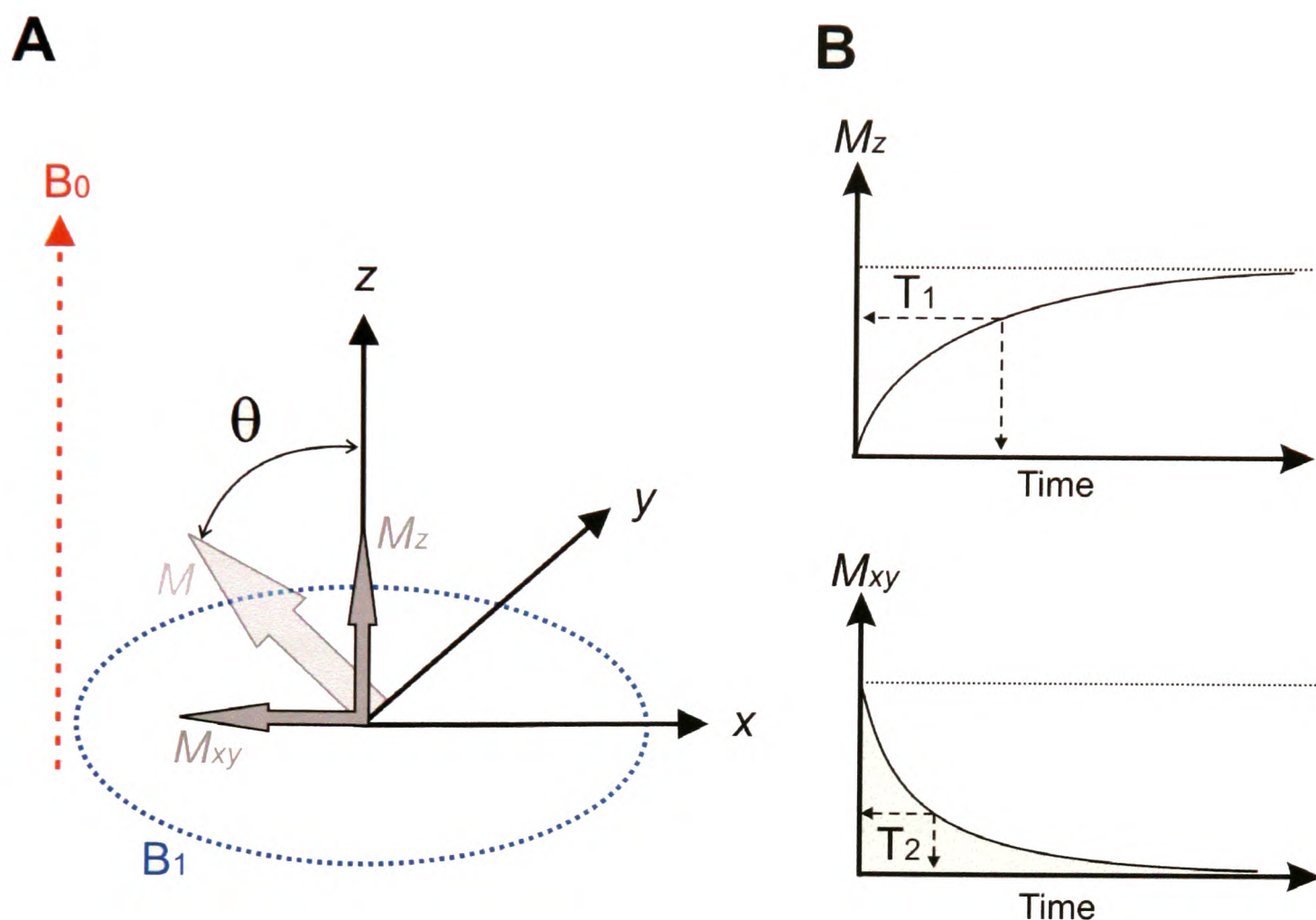


Figure 2.3: Relaxation times.

A The magnetisation vector M can be decomposed into a component M_z that lies parallel to the B_0 field and a second component M_{xy} that lies in the transverse plane. **B** The time it takes for M_z to return to 63% of its value before excitation is called T_1 (upper graph) and the time it takes for M_{xy} to decay to 37% of its value at equilibrium is called T_2 (lower graph).

The transverse (spin-spin) relaxation time on the other hand is characterised by the exponential decay of the transverse magnetisation M_{xy} following the excitation pulse and is measured by the time T_2 that it takes M_{xy} to decay to 37% of its equilibrium value. The reason for the decay of M_{xy} lies in the interaction between the spins of neighbouring nuclei. Immediately after the application of the excitation pulse, all spins precess in phase, thus creating the transverse component of magnetisation. However, the interactions between the individual spins create random local magnetic

field variations which cause fluctuations in the precessional frequency of the individual nuclei. This dephasing of the individual spins then leads to the decay of the net transverse magnetisation. Again, T2 relaxation times vary according to tissue type and are much faster than T1 relaxation (e.g., 110 ms for grey matter, 80 ms for white matter and > 2000 ms for cerebrospinal fluid at 3 Tesla)(Wansapura et al. 1999). Furthermore, interactions between spins are not the only components affecting the decay of transverse magnetisation. Slight inhomogeneities in the static B_0 field also lead to slightly different precession frequencies of nuclei in the sample. This results in additional dephasing of the spins, which in turn causes M_{xy} to return to equilibrium faster than T2. The decay caused by these field inhomogeneities is termed T_2^* . These field inhomogeneities have several origins: Firstly, the B_0 field may not be perfectly homogenous due to imperfections of the magnet. More importantly, the geometry and magnetic susceptibility of a sample affects the local B_0 . This effect is particularly severe in the boundary regions of tissues with differing magnetic susceptibilities, such as in the regions near the air-filled sinuses. The extremely steep differences in the B_0 field in these regions lead to a very rapidly occurring T_2^* relaxation, which in turn causes signal loss. This signal loss is particularly undesirable for fMRI studies of olfaction, since it affects the ability to image olfactory regions in the orbitofrontal cortex. However, T_2^* is also affected by the level of deoxyhaemoglobin in blood vessels and therefore forms the basis for blood oxygenation level dependent (BOLD) imaging (described below).

2.2.1.3 *Spatial encoding*

If we were to put a participant in a MRI scanner and apply a radio frequency (RF) pulse at the Larmor frequency, all H-nuclei would be excited by the pulse and the detected MR signal in the receiver coil would contain no information about the spatial origin of the signal. For the resulting image to contain spatial information, it is therefore necessary to encode the emitted signal so that its components can be related to the spatial location of the nuclei that contribute to them. In MRI this can be achieved by the combination of two processes: firstly, slice selection and secondly, spatial encoding of the signal within the selected slice. Both these processes rely on the same basic principle, namely that the Larmor frequency is dependent on the strength of the magnetic field. Consequently, if the local magnetic field is varied across space, the Larmor frequency will vary accordingly. The MRI scanner is equipped with gradient coils that are capable of generating magnetic field gradients in all 3 dimensions of space (G_x , G_y , G_z) in addition to the static B_0 field. A linear gradient along the head-to-toe direction of a person lying in the scanner (G_z) has the effect that every transverse slice will resonate at a different Larmor frequency. If an excitation RF pulse with a narrow bandwidth is then applied, only those nuclei in a slice where the Larmor frequency matches that of the applied pulse will absorb the pulse and generate a signal, thereby selecting the slice. Obviously, both the amplitude of the magnetic field gradient (G_z) and the bandwidth of the applied excitation pulse determine the slice thickness. Naturally, the slice selection gradient and RF excitation pulse do not generate any spatial information within the selected slice. To resolve this, a second field gradient (G_x) is applied orthogonally to the slice selection gradient while the signal is being received. This gradient causes the excited nuclei to precess at varying frequencies along the x-axis, thus encoding spatial information along one axis

of the imaged slice. This G_x gradient is termed read-out gradient or frequency encoding gradient.

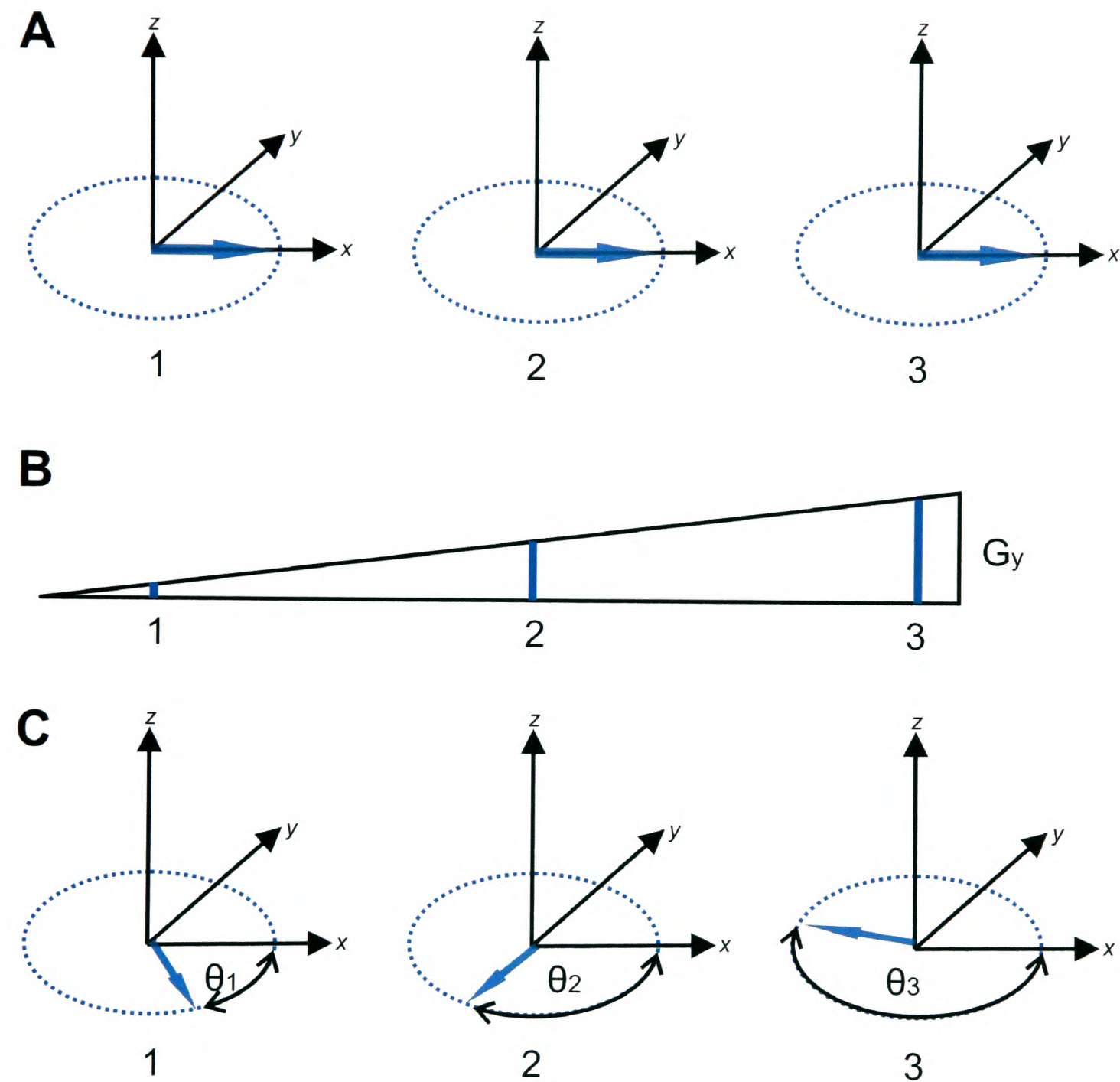


Figure 2.4: Phase encoding.

A After the excitation pulse, all spins precess at the same frequency and in phase. **B** The application of the gradient G_y causes the spins at different locations to precess at different frequencies. **C** After termination of G_y , the spins precess at the same frequency again, however their phases have been shifted by θ , depending on the strength of the previously applied gradient.

To complete the spatial encoding within a selected slice, a third field gradient (G_y) is applied prior to and orthogonally to G_x . This gradient G_y is termed the phase-encoding gradient and encodes spatial information as follows: after the application of the slice selection gradient and the appropriate excitation RF pulse, all spins in the selected slice precess in phase with each other at the same Larmor frequency. When the phase encode gradient is then applied, the spins will have their resonance frequency and therefore their rate of precession altered according to their position along the y-axis (Figure 2.4). After the phase-encoding gradient has been switched off, all spins along the y-axis will again precess at the same frequency, but their phases will be changed according to their position along the y-axis. Therefore, by manipulating both frequency and phase of the spins in a given slice, each spatial location is uniquely encoded. An image can then be reconstructed via a 2D Fourier transformation (described below).

In addition to measuring each slice separately, it is also possible to image an entire 3D volume with a single excitation RF pulse. Instead of the slice selection gradient, the spatial localisation in the third dimension is created through the application of a second phase encoding gradient perpendicular to the slice plane. This 3D or volume imaging has the advantage of a higher intrinsic signal-to-noise ratio (SNR), because the signal and noise are derived from the entire volume rather than a single slice. In contrast, 2D imaging only generates signal from a single slice but noise from the entire volume. The main disadvantage of 3D imaging is longer imaging time, due to the increase in phase-encoding steps.

2.2.1.4 *Image reconstruction and k-space*

With the method described above we can encode spatial information into our data set by selecting an appropriate slice with G_z , and then encoding each position in the remaining x-y plane by frequency (G_x) and phase (G_y). It is now necessary to decode the resulting signal into a spatially resolved anatomical image. The signal obtained by the receiver coil after the application of the excitation RF pulse and the various gradients is in frequency space, or k-space. Therefore, k-space consists of the raw data collected during the image acquisition and can be represented in a 2 dimensional coordinate system, with k_x corresponding to the time (integral) of the measurement gradient (G_x) and k_y corresponding to the phase encoding steps produced by G_y . Represented like this, k-space consists of all the image profiles acquired in one slice. The coordinates in k-space (k_x, k_y) at which data is acquired correspond to the frequencies of the sinusoidal waves that form the basis for the Fourier transform used to reconstruct the images. Fortunately, the information contained in the k-space image can be transformed into spin densities in Euclidian space by using Fourier transformation (Figure 2.5).

Obviously, the speed with which the entire k-space can be sampled determines the image acquisition time and therefore the temporal resolution of a functional imaging experiment. Several imaging sequences are available that sample k-space in different ways, two of which (echo-planar imaging and spiral imaging) will be described in more detail, because they have been employed in experiments described in this thesis.

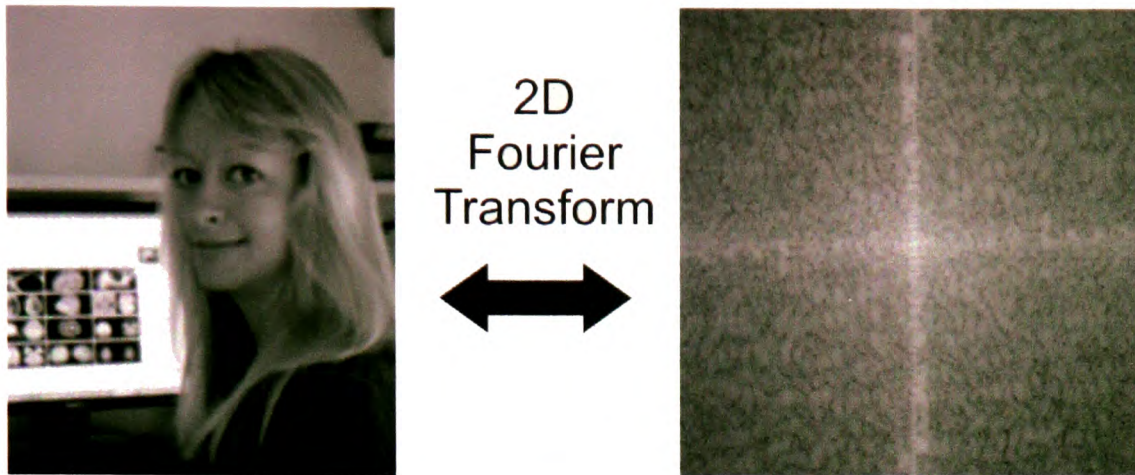


Figure 2.5: Fourier transform.

The figure shows how a spatially resolved image (left side) can be transformed into its constituting spatial frequencies (right side) using a 2D Fourier transform. Similarly, k-space images can be reconstructed using an inverse Fourier transform. The information content is identical in both images.

2.2.2 Echo-planar imaging

The most widespread technique of sampling k-space is that of echo-planar imaging (EPI), originally proposed by Mansfield (1977). Its particular usefulness lies in the fact that the complete k-space is sampled in a zigzag trajectory after a single excitation RF pulse, thereby dramatically reducing image acquisition time. This is achieved by repeated reversals of the read-out gradient (G_x) as well as application of multiple phase-encode gradients (G_y) to move the k-space trajectory along the y-axis (Figure 2.6). In modern MR scanners with high-performance gradient systems, it is possible to image about 25 slices with an in-plane resolution of about $3\text{mm} \times 3\text{mm}$ in approximately 3s. This makes it possible to observe changes in cerebral activity at relatively good spatial resolution, albeit with low temporal resolution (e.g. seconds). As described below, the comparatively low temporal resolution is not very problematic, since the observed changes in the MR signal are equally slow.

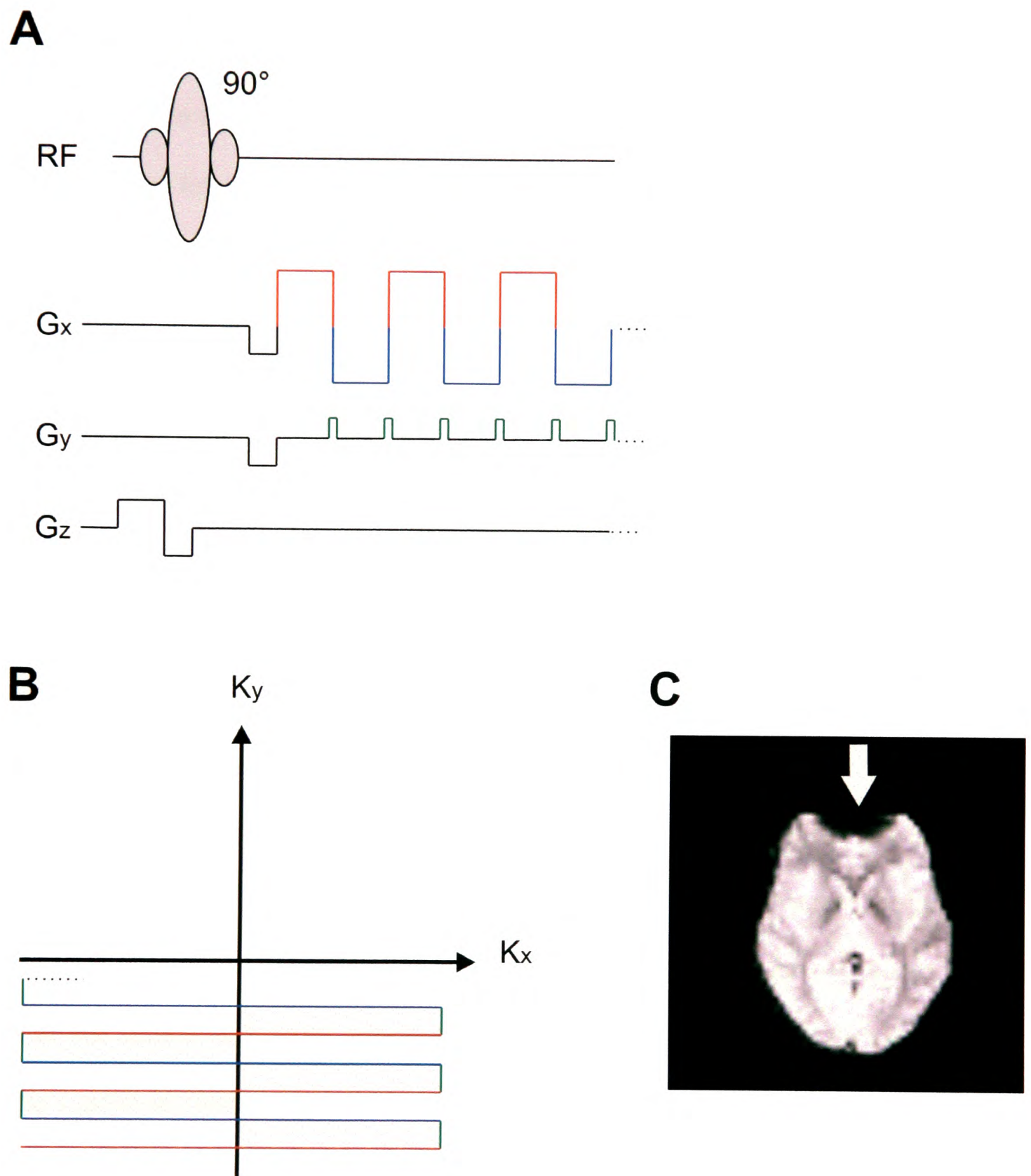


Figure 2.6: Echo-planar imaging

A The EPI pulse sequence comprising of the excitation RF pulse, the slice selection gradient G_z and the G_x and G_y gradients that spatially encode information within each slice. **B** The k-space trajectory of the EPI sequence. **C** A spatially resolved axial slice obtained after reconstruction from k-space. Note the signal dropout (white arrow) in the frontal brain regions due to susceptibility effects.

It should be noted that the necessary switching of G_x is relatively taxing on the gradient system and, in addition, the rapidly switching gradients can cause peripheral nerve stimulation in humans. Both the hardware and physiological limits of EPI have been approached in modern imaging systems and it is likely that further reductions in image acquisition time will rely on novel imaging sequences. One particular problem with EPI sequences is their vulnerability to artefacts resulting from imperfections of the magnetic field that are especially pronounced near air-tissue boundaries. Such field inhomogeneities can result in signal losses and geometric distortions particularly in the regions of the orbitofrontal cortex, a problem greatly affecting fMRI studies of olfaction.

2.2.3 Spiral imaging

An alternative approach to EPI that also rapidly samples k-space is that of spiral imaging (see Block and Frahm (2005) for a recent review). As the name suggests, the k-space trajectory follows an equidistant spiral path in k-space (Figure 2.7) starting either at the centre of k-space (spiral-out) or at the outside borders and spiralling inwards (spiral-in) using specialised sinusoidal gradient waveforms (Glover 1999). The main advantage of this technique is that it is less taxing on the gradient system and that all sampled data is used to reconstruct the final image, resulting in extremely fast and efficient image acquisition. However, the fact that data is not sampled in a Cartesian grid necessitates reconstruction of the dataset into conventional Cartesian space (regridding) before a Fourier transform can be applied, which is computationally demanding.

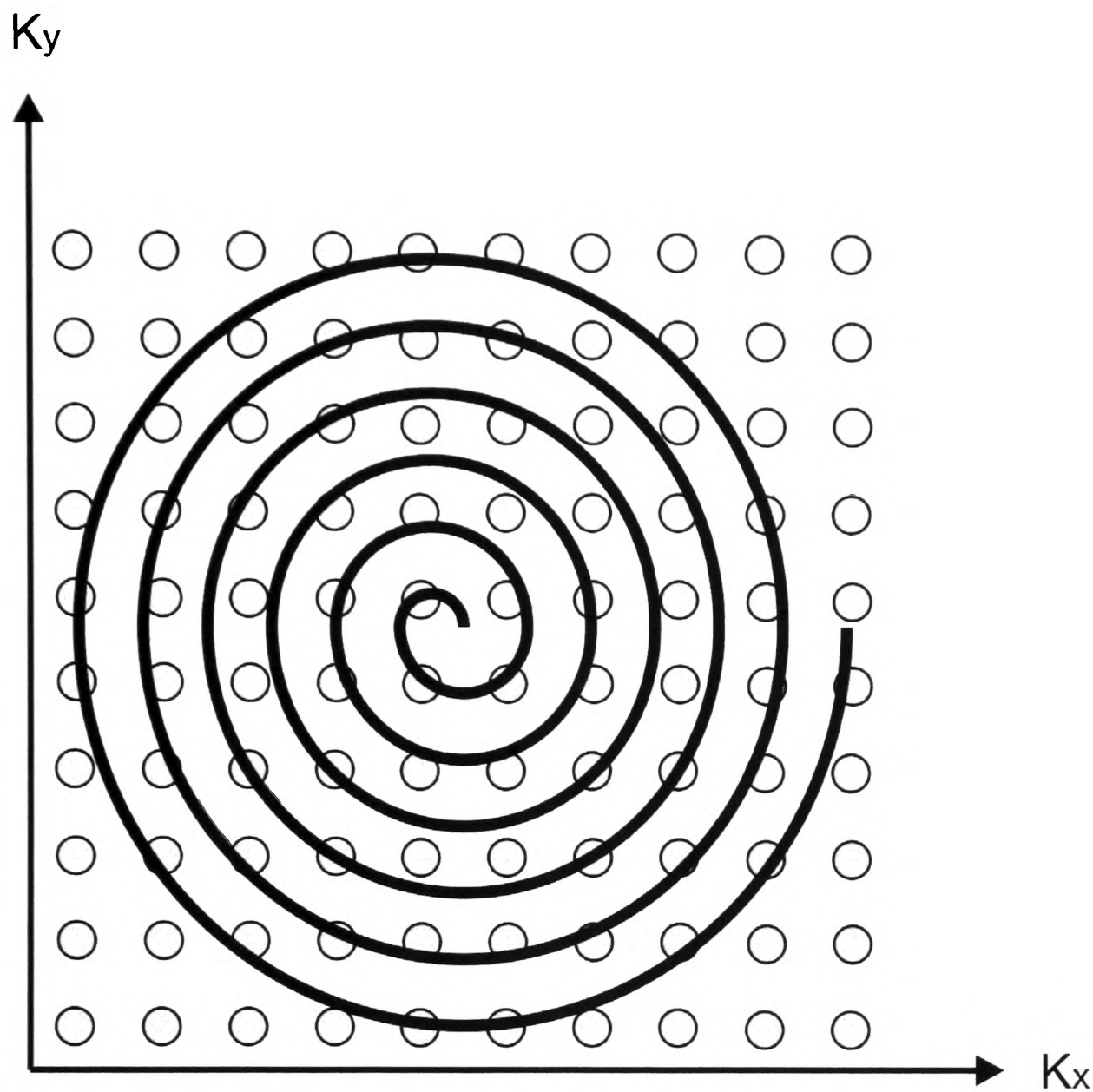


Figure 2.7: Spiral trajectory.

Another approach to mapping k-space is achieved by spiral imaging. Note that the angular velocity with which the path is traced needs to vary in order to avoid oversampling of the central aspects of k-space and undersampling the periphery.

2.2.4 Sensitivity Encoding (SENSE)

The novel technique of sensitivity encoding (SENSE) allows for extremely fast image acquisition (Pruessmann et al. 2001; Pruessmann et al. 1999) by utilising the sensitivity properties of multiple receiver coils used in parallel. Because spatial information is encoded by the relative sensitivity of each of the coils, this reduces the number of phase-encoding steps required to sample k-space, which in turn reduces the

total acquisition time. It should be noted that the reduction in acquisition time by a factor R leads to a decrease of the signal-to-noise ratio (SNR) by a factor of $\sqrt{R}$, leading to a trade-off between speed and image quality. However, in the case of imaging the OFC the improvements obtained by the faster acquisition surpass the decrease in SNR, thereby making this technique a feasible alternative to gradient-encoding methods (see Chapter 3).

2.2.5 Susceptibility artefacts

One of the major challenges facing fMRI of olfaction is the fact that higher-order olfactory processes appear to be located within the OFC. Due to the proximity of this region to the air-filled sinuses, the area is particularly affected by susceptibility artefacts (Ojemann et al. 1997) that mainly consist of geometric distortions and signal loss (Figure 2.8). For the purpose of this thesis it is therefore paramount that all imaging parameters are optimised in order to curtail these effects. Several approaches can be used to minimise the extent of the artefacts. The use of localised shimming methods for example can counterbalance the effects to some degree (Wilson et al. 2002a). Additionally, specialised preparation gradients may be applied that partly compensate for the susceptibility gradients (Deichmann et al. 2002). The major disadvantage of this approach is that it lengthens the imaging time. The use of very short echo times (Gorno-Tempini et al. 2002) as well as a slightly oblique acquisition angle (Deichmann et al. 2003) can further improve image quality, without altering acquisition time. To a certain extent, a map of the static B_0 field can also be used to undistort the functional images after acquisition (Jezzard and Balaban 1995). However, this approach redistributes the shifted signal to its plausible spatial origin

and as such does not alter the intrinsic signal obtained from a given brain region. As described in detail in Chapter 4, the use of a passive shim (Wilson et al. 2003; Wilson and Jezzard 2003) may be one possible way to improve image quality in OFC that does not necessitate specialised pulse sequences or extensive post-processing of the data.

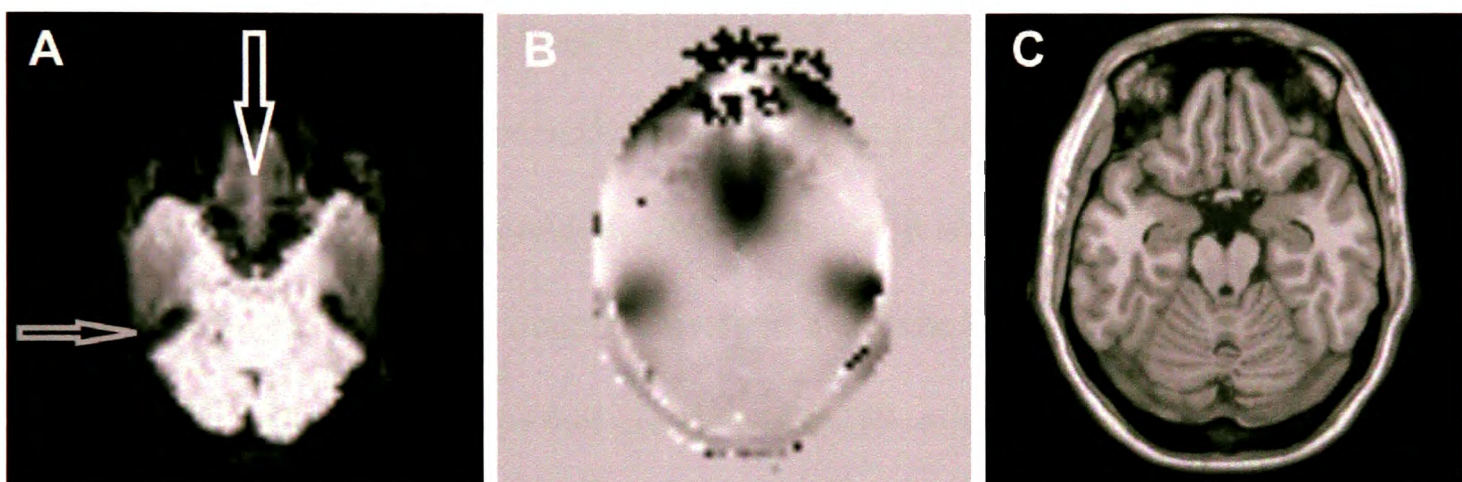


Figure 2.8: Susceptibility effects.

A The axial EPI image clearly shows areas of low signal due to susceptibility effects both in the OFC (white arrow) and in the temporal lobes (grey arrow). **B** Corresponding map of the static B_0 field. **C** High resolution anatomical image of the same region for comparison.

2.2.6 Blood Oxygenation Level Dependent (BOLD) Imaging

2.2.6.1 *Basic principles of the BOLD contrast*

The main principle underlying the technique of fMRI is that neural activity in the brain requires delivery of oxygen and glucose via the vasculature thereby increasing regional cerebral blood flow (Sokoloff 1977). A very useful property of the haemoglobin (Hb) molecules that transport oxygen within in the red blood cells in the

human body is that oxygenated haemoglobin is diamagnetic and deoxygenated haemoglobin is paramagnetic. This results in both types of haemoglobin possessing different magnetic susceptibilities, which makes it possible to use them as an intrinsic contrast agent for brain imaging studies. As described above, the magnetic susceptibility of a given substance affects the T_2^* decay of the transverse magnetisation. Therefore, the small magnetic field distortions caused by deoxyhaemoglobin within and around the blood vessels produce a slight alteration in the local MR signal that can be detected with T_2^* weighted contrast. This effect was first described by Ogawa and colleagues (1990) and the resulting contrast was named blood oxygenation level dependent (BOLD) contrast and forms the basis of fMRI. As a consequence of increased neural activity in a certain area of the brain, the local cerebral blood flow (CBF) to that area increases to satisfy the heightened demand for oxygen and glucose. However, the CBF increases the delivery of oxygen much more than the cerebral metabolic rate of oxygen consumption ($CMRO_2$), resulting in a change in the ratio between oxygenated Hb and deoxygenated Hb (more oxygenated and less deoxygenated Hb) in the area of neural activity (Fox and Raichle 1986). This in turn leads to a reduction of the magnetic field susceptibility and a local slight increase in MR signal intensity (Figure 2.9). These BOLD changes in the MR signal following neuronal activity have been termed the haemodynamic response (HDR). Typically, the observed increase in MR signal is in the region of 2-5% from baseline, which only slightly exceeds the intrinsic 'noise' fluctuations. The magnitude of the increase is dependent on the area of cortex imaged and the strength of the static magnetic field, with higher fields resulting in stronger signal (Kastrup et al. 2002; Turner et al. 1993).

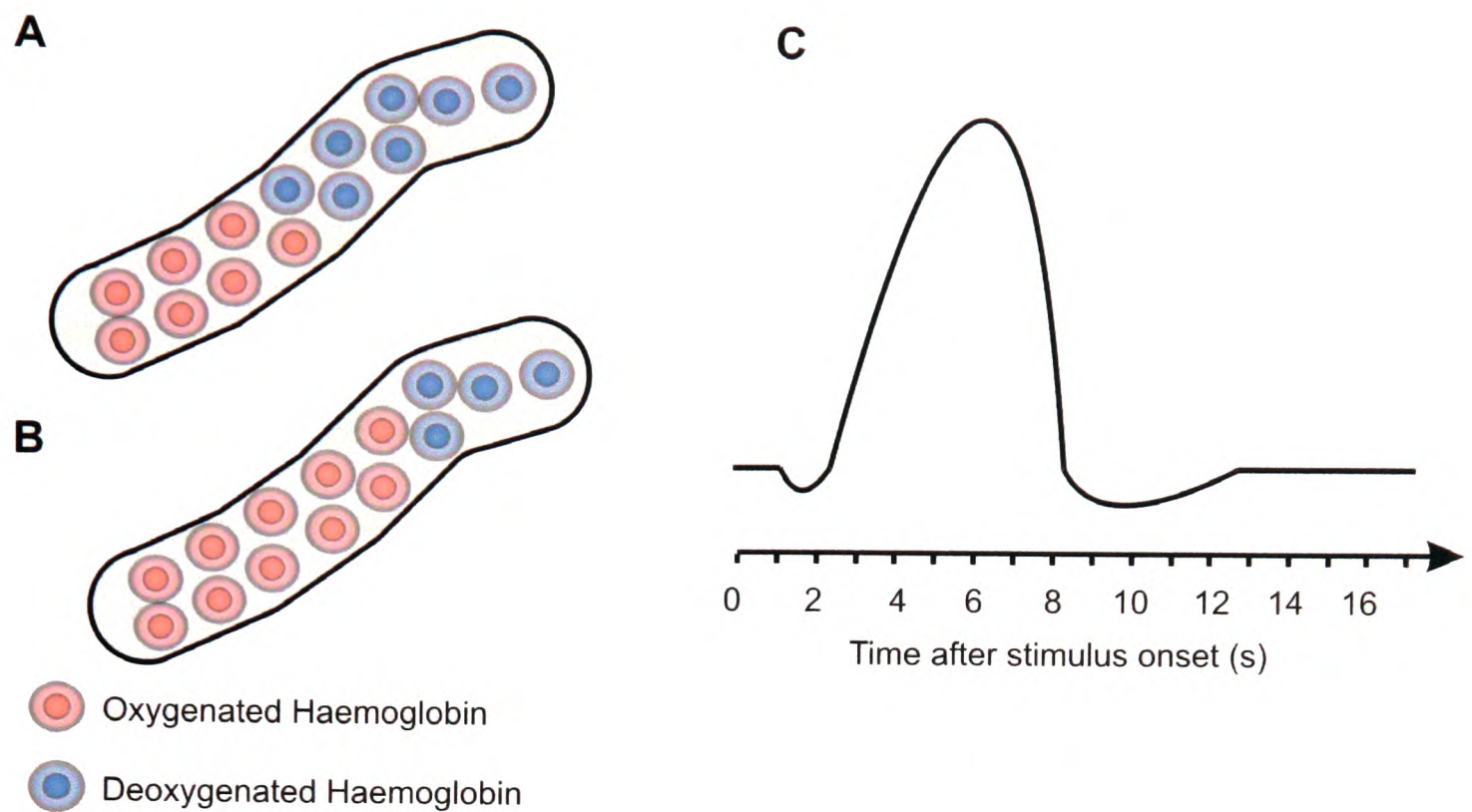


Figure 2.9: BOLD response.

A The baseline state of blood oxygenation in a blood vessel. **B** Neuronal activation leads to an increase in blood flow and to increased levels of oxygenated haemoglobin in the vessel. **C** This increase in oxygenated Hb causes a positive MR signal. Note that the positive signal is preceded by a small negative dip and is followed by a prolonged negative undershoot before return to baseline.

It should be noted that the precise relationship between neuronal activity and the BOLD response is still not fully understood. However, recent studies reported that the BOLD response correlates best with the mean local field potentials (LFPs) but not with the output spiking activity (Lauritzen and Gold 2003; Logothetis et al. 2001). Since LFPs are thought to measure synaptic events, these findings suggest that it is the afferent input and intracortical processing rather than efferent output that drives the haemodynamic response.

2.2.6.2 *Spatial characteristics of the BOLD response*

Since BOLD imaging is an indirect measure of neuronal activity, one of the fundamental assumptions of fMRI is that the haemodynamic response is spatially co-localised with the activity of neurones. Therefore, the theoretical limit of the spatial resolution would be the size of the smallest capillaries contributing to the signal change (Menon et al. 1998). The smallest capillaries found in the cerebral cortex are typically less than a millimetre in length and are separated by a distance of approximately 100 microns, causing the maximum spatial resolution of fMRI to be around this order of magnitude (Zarahn 2001). However, various larger sources influence the measured signal, particularly the supply arteries and draining veins. Neural activity in a given brain region causes an increase in arterial blood flow in larger vessels that can be more than a centimetre away from the site of activity, which can lead to substantial MR signal changes that are not co-localised with the neuronal source. The MR signal changes around large vessels can be as much as 10 times larger than those produced by the capillaries within grey matter (Gati et al. 1997). These large vessel effects further limit the spatial resolution of fMRI investigations.

In a typical fMRI experiment with whole brain coverage, data is acquired from a voxel with side lengths of a few millimetres. In neuronal terms, this is a large area, containing millions of nerve cells, glia and other types of tissue. A resulting problem is that of partial volume effects, because the MR signal measured at a given voxel is the summed signal from all types of underlying tissue. Therefore, in addition to the active neurones and the local capillary bed, there may be other types of tissue present that do not contribute to the measured activity. This makes it particularly difficult to quantitatively compare the obtained signal between different voxels, because the observation that 2 voxels generate identical signal does not necessarily reflect

identical underlying neuronal activity. These effects are especially pronounced on the edge of the brain, where one voxel can contain grey matter, white matter and cerebrospinal fluid.

2.2.6.3 *Temporal characteristics of the BOLD response*

The temporal resolution of fMRI is not primarily constrained by the ability to rapidly acquire images, but rather by the temporal characteristics of the haemodynamic response. Whereas the neuronal events happen in the millisecond range, the resulting vascular response takes several seconds to occur. The measurable haemodynamic response function (HRF) has some typical characteristic across brain regions (Figure 2.9 C). A small initial dip of the signal has been observed in some studies that appears 1-2 seconds after the onset of stimulus presentation (Buxton et al. 1998; Frahm et al. 1996). This dip corresponds well with the transient increase of deoxyhaemoglobin that has been detected using optical imaging (Malonek and Grinvald 1996). The authors of this study speculated that the cause of the initial dip was a temporary uncoupling between CBF and CMRO₂ to the effect that increased metabolic demand following heightened neural activity caused a decrease in vascular oxygen concentration before blood flow increased to satisfy this demand.

This initial dip is then followed by an increase in the MR signal commencing approximately 2s after stimulus onset and peaking after 6 seconds (Bandettini et al. 1992). The response then reaches a plateau that cannot be exceeded even by sustained stimulation. Several seconds after stimulus offset, the response returns back to baseline. Frequently, a post-stimulus undershoot that can last for up to 30s, depending

on the length of the sensory stimulation can be observed, before the signal returns to baseline (Buxton et al. 2004).

It should also be noted that quantitative interpretations, particularly of the height of the haemodynamic response should be approached with caution, since the precise relationship of neural activity and HR are still relatively poorly understood. One complicating feature of the HR is its non-linearity, e.g. the summed response to several short stimulations does not equal the response to one long stimulation (Birn et al. 2001). This non-linearity is particularly pronounced for very short stimuli (less than 4 seconds) where the elicited response is of higher amplitude but shorter duration than predicted by linear summation. Additionally the relationship between stimulus duration and HR appears to vary to a great extent depending on which region of cortex is imaged. As such, these effects are of relatively minor importance for ‘block designs’ with stimulus durations of about 30s but become extremely significant for event-related paradigms (described below).

2.2.7 Paradigm design

2.2.7.1 *Block paradigm*

The first and still most common paradigm for stimulus presentation in a fMRI experiment is the block paradigm. Here, a series of trials are presented repeatedly over a fixed time period, typically in the region of 30s. This activation period is then typically followed by a rest period of similar time without any stimulation, which allows for the BOLD response to return to baseline and for the collection of data in the absence of any task. The two resulting experimental conditions (task and control)

can then be statistically compared using a subtractive approach. Whereas brain regions involved in the task will display increased neural activity and therefore an increase in the detected MR signal, during the baseline condition (i.e., in the absence of a task), the MR signal will fluctuate randomly in these regions. The subtraction of data collected during the baseline period from data collected during the activation period can then reveal brain regions involved in the task. The main advantages of the block paradigm are that they are easily created and analysed and that they have the strongest statistical power to detect cerebral activation. However, because of the rapid habituation of the olfactory system to sustained stimulation, block designs are not a feasible option for the study of olfactory processing and it is therefore necessary to use the event-related designs described below.

2.2.7.2 Event-related paradigm

Event-related paradigms differ from block-designs in that they present single stimulus events in rapid succession and do not rely on the HR to return to baseline before the next stimulus is presented (Buckner et al. 1998; Rosen et al. 1998). These designs have been made possible by the observation that even extremely short stimulus presentations (under 1s) elicit a measurable BOLD response (Konishi et al. 1996). For many cognitive and perceptual experiments these rapid event-related designs have the advantage of limiting stimulus predictability and habituation by interleaving trial types and varying the inter-stimulus-intervals. In a typical event-related fMRI study several conditions (tasks or stimuli) which are short in duration are presented in random order, with interstimulus intervals as short as 1.5s (D'Esposito et al. 1999; Rosen et al. 1998). Consequently, these paradigms rely on very rapid image

acquisition methods. Additionally they require more sophisticated analysis strategies, because the HRF of several stimulations overlap. A major assumption for the use of event-related fMRI is that of the linear summation of the HRF from successive trials (Buckner 1998) which is not necessarily always the case (Miezin et al. 2000).

2.3 IMAGE ANALYSIS

A large amount of image processing and data analysis is required to arrive at the familiar colour-coded brain activation maps from the raw data acquired by the MRI scanner. All the data presented in this thesis were analysed using FMRIB's Software Library (FSL), which has been developed at the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (Smith et al. 2004). The following section describes the processing steps involved.

2.3.1 Data reconstruction and slice timing correction

Typically, the raw data gathered from the scanner consists of a time-series of a few hundred T2* weighted images in k-space, sampled at a rate of 2-3s during the course of an experiment. As described above, the k-space data first needs to be decoded to obtain a spatially resolved image using an inverse Fourier transform. After reconstruction, some additional pre-processing is usually necessary in order to prepare the data for statistical analysis. Particularly for event-related designs it is necessary to apply some sort of slice acquisition time correction. The reason for this is that not all the slices comprising one volume are collected at the same time. This means that there will be a few seconds of time difference between the acquisition of the first and the last slice. To correct for these timing errors, the timing of each voxel within a given slice has to be slightly shifted in time so that the corrected slices all appear to have been collected simultaneously.

2.3.2 Motion correction

The major problem at the pre-processing stage is that of head motion. Because the later statistical analysis essentially constitutes the analysis of the MR signal of a voxel over time, it is extremely important that the volume of brain contained in a single voxel remains constant. However, it is inevitable that participants generate some form of head movements during the course of an experiment. Breathing for example introduces regular head movements that need to be corrected since these can introduce serious artefacts in the activation maps, an issue that is extremely problematic for olfactory research, because inspiration and the ability to smell a stimulus are essentially time-locked. This has the effect of producing stimulus-correlated motion, i.e. head movements that occur simultaneously with the stimulus presentation. Predominantly, this can then result in artefactual activation in brain regions near tissue boundaries at the edge of the brain and around the ventricles, which exhibit large intensity transitions of the MR signal. Due to the partial volume effects described above, even slight movements in the order of a few millimetres can significantly alter the intensity of the MR signal. If these changes occur simultaneously with the stimulus presentation, they become virtually indistinguishable from the changes produced by the BOLD response. It is therefore necessary to realign the images prior to image analysis and to visually inspect the results for signs of motion artefacts.

The correction algorithm used in this thesis is MCFLIRT (Jenkinson et al. 2002; Jenkinson and Smith 2001) that realigns each volume to a template volume acquired halfway through the experimental run using rigid body transformations (Figure 2.10). This approach assumes that head motion will cause either a translation

or a rotation of the head, but will not change the shape of the image (even though pulsatile motion within the brain would do exactly that).

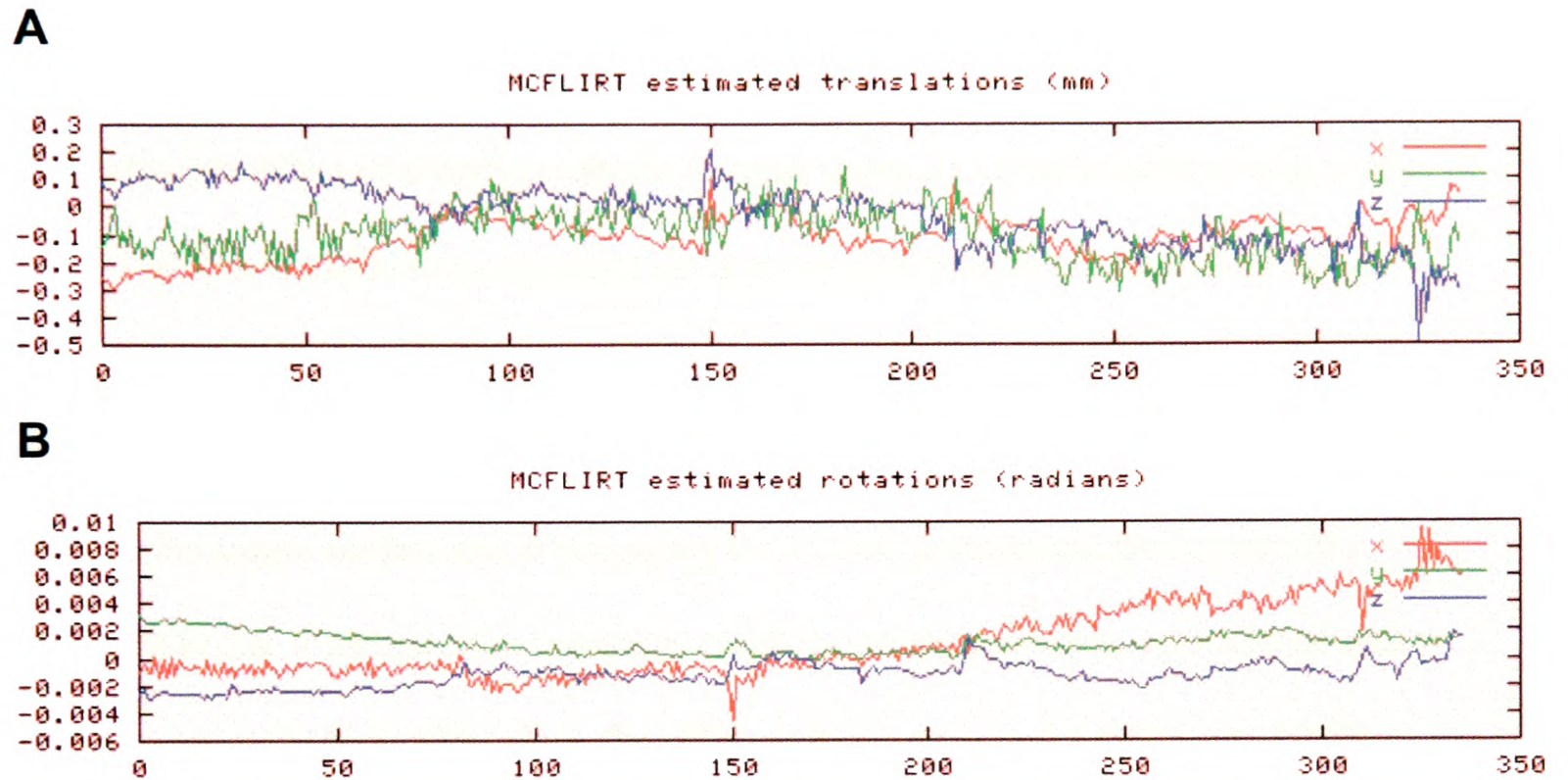


Figure 2.10: MCFLIRT motion correction.

A Example from a single subject of the estimated translational motion in all 3 dimension and **B** the estimated rotation along all 3 axes. All images were realigned to a template image acquired half way through the imaging run.

2.3.3 Spatial and temporal filtering

After having realigned the data set using a motion correction algorithm, several other image processing steps need to be taken before statistical analysis can be carried out. All collected volumes undergo spatial smoothing, which is essentially a blurring of the images using a Gaussian filter function of a set width, expressed as full width at half maximum (FWHM). Spatial smoothing increases the SNR, because random noise from neighbouring voxels should cancel each other out. However, the

filter width should be small compared to the expected spatial extent of the activation, because it would otherwise also reduce the signal. A second reason for applying spatial smoothing is that for the later statistical analysis to be valid, it is required that the data has been smoothed (Friston et al. 1994).

A second processing step of intensity normalisation is also applied to the data, which has the effect of transforming the average intensity in each volume to the same level. This has the effect of cancelling out any overall shifts in the measured signal intensities that are due to scanner drifts or other external factors. The reason for this is that intensity differences can significantly alter the subsequent statistical analysis, both on the single subject and at the group level. One problematic factor with this approach is that if an activation paradigm leads to strong activation over a large area, intensity normalisation will reduce the effect in these areas and might even lead to bogus ‘deactivation’ in non-activated areas. An alternative method to overcome these problems is to scale the entire imaging run of each subject to the same overall mean (grand mean normalisation), an approach used for the data presented in this thesis.

After spatial smoothing and intensity normalisation, the time series data of each voxel undergoes a process of temporal filtering to further reduce the noise in the dataset. There are two possible types of temporal filtering, high-pass and low-pass. The high-pass filtering removes all slowly varying signals from the data, such as those caused by scanner drift or cardio-respiratory effects. When setting the parameters of the temporal filter, it is extremely important to set it higher than the repeat time of the experimental stimulation. Failure to do so would cause variations induced by the experiment to be filtered too, which is clearly undesirable. For all experiments presented in this thesis, high-pass temporal filtering has been applied at twice the time of the longest rest-stimulation period. The autocorrelation of each voxel is then

calculated (pre-whitening) and accounted for in the later statistical analysis (Woolrich et al. 2001). Without pre-whitening, there would be a danger of overestimating the number of actually active voxels, because autocorrelations between neighbouring voxels essentially reduce the number of truly independent samples.

2.3.4 Statistical analysis of fMRI data

The statistical analysis of the dataset after the pre-processing state ultimately tests for a signal that is varying together with the experimental paradigm. This is done by modelling the BOLD responses over the course of the experiment with a general linear model (GLM) for each voxel, which in its simplest form can be written as:

$$y = \beta x + \varepsilon$$

where y is the signal amplitude over time, x is the explanatory variable (EV), β the associated parameter estimate (PE) and ε the error.

For all data in this thesis the haemodynamic response function (HRF) was modelled as a set of two gamma functions whose peaks have been shifted by 6s with respect to the onset of stimulation to account for the temporal lag of the BOLD response. For each voxel the PE and its variance were then derived by the GLM analysis, which allowed for an estimate of the amplitude of the response to the stimulation in this voxel. With these 2 parameters, a t-statistic can easily be calculated for each voxel by dividing the PE by its standard error (which is the square root of the variance). These t-statistic maps are then converted into Z-statistic maps that are independent of degrees-of-freedom and can be associated with a corresponding probability value (p-value). The activation for different experimental conditions can

then be calculated by the contrasts of the various PE (COPEs) of the explanatory variables.

Because there are several thousand voxels in a typical volume of the brain, it is necessary to correct the statistical threshold for multiple comparisons, to avoid false positives. For example, if the p-value of 0.05 is used, then 5% of non-activated parts of the brain would show false-positives. The most widely used statistical method of Bonferroni correction, which adjusts the p-value to account for the problem of false positives, is overly conservative since it is assumed that all measures are independent from each other. As described above, the data is smoothed prior to analysis and therefore contains less statistically independent elements than voxels. The number of independent resolution elements (resels) can be approximated using Random Field Theory (Worsley et al. 1998; Worsley et al. 2004). This approach gives a less conservative p-value than Bonferroni correction and avoids the problem of false-negatives (i.e. failure to detect truly activated voxels). In all the data presented in this thesis a cluster detection approach to statistical testing has been used (Friston et al. 1994) which is based on Random Field Theory but also takes into account the spatial extent of an activation cluster. In this approach, the statistic is first thresholded at a certain z-value and then the p-value for each of the remaining clusters is calculated based on their size. The spatial extent of a cluster clearly contains important information, since with increasingly larger clusters, the likelihood of it being generated solely by false-positives decreases.

Because the inferences drawn from an fMRI study mainly concern the population rather than an individual, another set of statistics needs to be applied to calculate activation maps for a group of subjects. Since the size and shape between individual brains can vary considerably, it is necessary to transform these individual

brains into a standard frame of reference before applying group level statistics. All data presented here is reported in the co-ordinates of the canonical Montreal Neurological Institute (MNI) standard brain which reflect the average of 152 participants, with the necessary transformations having been computed using FLIRT (Jenkinson and Smith 2001). A mixed-effect multi-level GLM (Beckmann et al. 2003) is then applied to the output of the combined parameter estimates from the individual subject analyses, taking into account the variability between subjects as well as the within-subject variance. This approach is more valid than a simple fixed-effect analysis, where the results of the group statistics may be biased by outliers, i.e. single subjects with uncharacteristic response patterns.

Modelling the height of the BOLD response in the GLM according to participant's behavioural ratings is not without its own problems. It obviously relies on cooperation of a participant, but more importantly it limits the analysis of the data to previously determined hypothesis without the possibility of explorative analysis. To address these issues, novel model free analysis techniques have been recently developed. Particularly the method of independent component analysis (ICA) is a promising new data exploration algorithm which has been successfully applied to fMRI data to identify resting state networks (Damoiseaux et al. 2006). The decompositions made by ICA can simultaneously extract a variety of different coherent resting networks and separate these from signal changes induced by head motion or other physiological noise, such as cardiac or the respiratory motion, which also makes it useful as a filter for data containing a lot of physiological noise.

2.4 MR-COMPATIBLE OLFACTOMETER

2.4.1 General requirements for an olfactometer

In order to deliver olfactory stimuli orthonasally in the MR scanner in a temporally accurate way, a computer-controlled olfactometer was designed. Previous reports on the design of olfactometers suitable for the MR environment were used as a guideline (Kobal 1985; Sobel et al. 1997). The olfactometer was intended to adhere to the following basic conditions for olfactory experiments:

- 1) during rest periods, the subject has to breathe from an odourless air source of known composition
- 2) during olfactory stimulation periods, the subject has to breathe odorised air of known concentration for a definable period of time
- 3) both odorised and odourless air should be of the same humidity and temperature in order to avoid imaging a thermal rather than an olfactory response
- 4) tactile stimulation due to the air flow should be eliminated or at least held constant over odour and non-odour phases of the experiment so as to avoid imaging a tactile rather than an olfactory stimulation

The olfactometer should thus be able to deliver a stream of either odorised or odourless air to a person lying in the scanner and should do so with high temporal precision and without providing any cues that could alert the person to the onset of the olfactory stimulation. Additionally, no ferromagnetic components of the olfactometer are allowed to be placed inside the scanner room, since they would constitute a safety hazard because of their inherent attraction to the magnetic field. Furthermore, all

electronic parts of the olfactometer have to be located outside the scanner room, since their operation would introduce image artefacts.

2.4.2 Orthonasal olfaction

In order to comply with the above demands for stimulus delivery, a computer-controlled olfactometer was designed, which allowed for the delivery of either odourised or odourless air for a pre-defined period of time (Fig. 2.11).

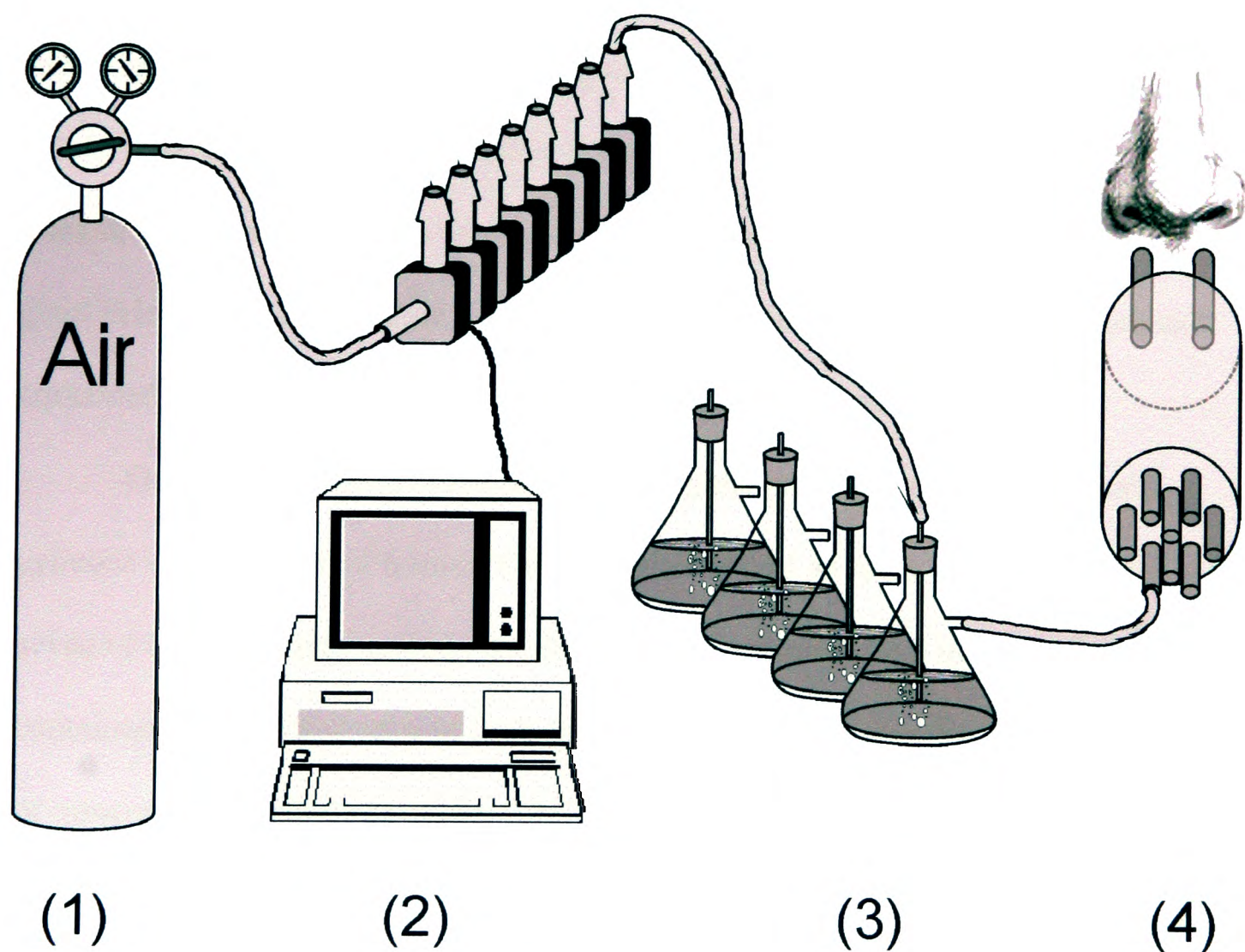


Figure 2.11: Sketch of the olfactometer.

(1) Medical air from the G-cylinder is delivered to (2) the computer-controlled valve system where the stream of air is directed into one of eight lines. From there, air runs to (3) the Erlenmeyer flasks where it is odourised by bubbling through a liquid containing the dissolved odorant. Finally, all lines from the flask converge in (4) the 'nose piece', a Teflon cylinder that has 2 outlets from where the air is delivered to the subject's nostrils. For illustration purposes, only one odour line is depicted.

The set-up of the olfactometer was as follows. A G-cylinder -placed inside the control room- containing pressurised medical air (BOC, UK) and equipped with a twin flow-meter was used as an air supply. The stream of air was delivered at a flow rate of 6 l/min via 2m of tubing that was connected to a valve system that was also located in the control room. To humidify the air, a 250 ml Buchner flask (Pyrex, UK) containing 100ml of water at room temperature was connected into the line from the cylinder so that all air was 'bubbled' through the flask before reaching the valve system. The valve system consisted of a custom built array of eight 12V solenoid valves (ACL Italy) and could be automatically controlled by a computer program. The valves were used to direct the airflow from the G-cylinder into either one of eight lines of tubing which in turn were individually connected to eight different 100ml glass Erlenmeyer flasks (Pyrex, UK) that were placed inside the scanner room and contained both the dissolved odorants and an odourless control solution.

Odorised air was generated by bubbling the pressurised air from the air cylinder at a flow rate of 6 l/min through either one of an array of eight flasks. Up to seven of the flasks may contain the dissolved odorant and the remaining flask contained an odourless solvent such as water. Odorants were either dissolved in water or mineral oil (Diethyl phthalate; CAS number 84-66-2), which itself has minimal odour. Each flask was closed by a two-hole (6mm diameter) silicone bung, through which a 10cm hollow soda lime glass rod was inserted. The outer end of the glass rod was attached to the valve system by 6m of Teflon coated tubing and served as the inlet. The other end of the glass rod was placed below the odorised liquid, so that the air stream could bubble through the liquid, thereby odorising the air. The other hole in the bung functioned as an outlet and was connected by 2m of tubing to the 'nose

piece'. The array of flasks was placed just outside the scanner approximately 2m away from the subject's head.

The 8 tubes from the flasks all converged in the specially designed 'nose piece' made of a Teflon cylinder with a diameter of 4cm and a height of 6cm. The cylinder had 8 holes on one end allowing for the 8 lines from the flasks to be inserted and 2 outlets which could be placed directly under the nostrils of the participant.

This set-up ensured a minimum time lag between the injection of odourised air and its delivery to the participant. Additionally, no auditory, thermal or tactile cues could alert the participant to the onset of the olfactory stimulus. The temporal lag between the switching of the valve system and the delivery of odours to the nose of a participant was approximately 250 milliseconds (this calculation is based on a tubing volume between bubbler and nostril of 25ml and a flow rate of 6 litres/minute). This lag was deemed acceptable for fMRI investigations, since the BOLD response itself has a lag in rise time of about 6s and is only sampled every 2-3s. However, compared to the Kobal olfactometer (which has a lag time of 20ms) the present system is rather slow.

2.4.3 Retronasal olfaction

For the two studies in this thesis requiring delivery of either a gustatory stimulus (Chapter 3) or a retronasally delivered olfactory stimulus (Chapter 5), a delivery system similar to that employed by other researchers was used (Cerf-Ducastel and Murphy 2001; de Araujo et al. 2003a; Kringelbach et al. 2003). The system consisted of a plastic bottle containing the dissolved smell or taste stimuli (2.12). A syringe system attached to a 2-way valve could be operated so that the

syringe was filled with the liquid contained in the bottle. Depressing the syringe then delivered the liquid into a Teflon tube placed in a subject's mouth. For accurate timing of the manually operated device, a visual 'countdown' was displayed to the operator of the device (which was not visible to the participant of the experiment), in order to alert him to the onset of stimulation.

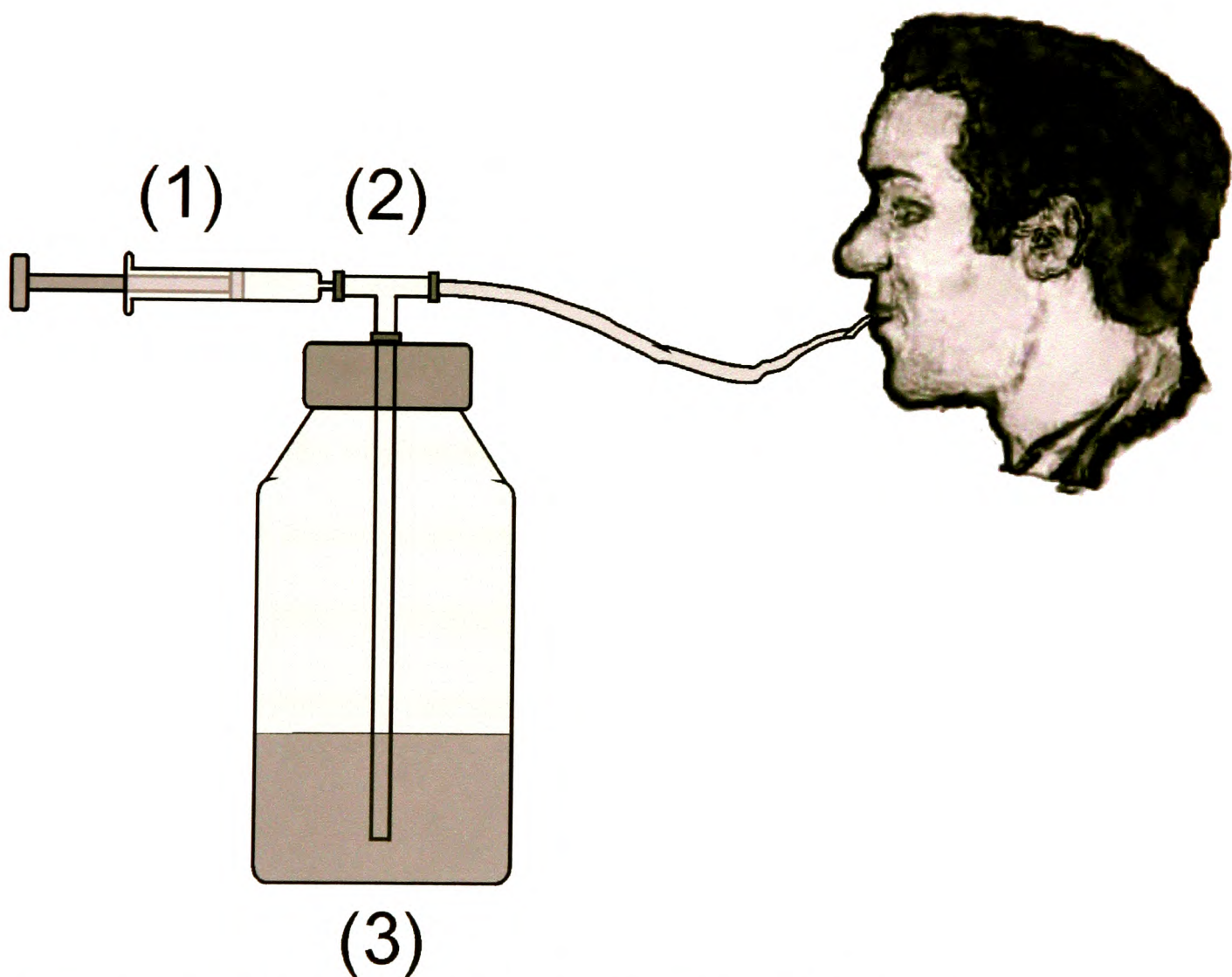


Figure 2.12: Sketch of the liquid odorant/taste delivery system.

A syringe (1) attached to a 2-way valve could be operated so that a given quantity of liquid contained in a plastic bottle (3) could be injected into a subject's mouth.

2.4.4 Effects of respiration on fMRI signals

Respiration can produce serious confounds in fMRI studies of olfaction since stimulus perception is correlated with inspiration, leading to stimulus correlated motion. It has also been reported that sniffing alone (in the absence of an odour) produces activation in the cerebellum (Sobel et al. 1998a; Sobel et al. 1998b).

A pilot fMRI study was therefore conducted to investigate the effects of respiration in olfactory areas. Since the presentation of the olfactory stimuli is time-locked to respiration, it is necessary to know whether respiration alone can lead to an increased BOLD response in the piriform cortex or olfactory areas within the orbitofrontal cortex. Additionally, the presence of an odorant might alter the respiratory pattern. For example subjects might take in deeper breaths whilst an odorant is present, especially if the intensity of the odour is low or the odour is very pleasant. Conversely, unpleasant odours may induce a very shallow breathing response in order to minimise exposure. In this experiment, subjects were cued to take a deep breath in order to simulate a pattern of altered breathing, since this is likely to cause significant stimulus correlated motion.

2.4.4.1 *Subjects and paradigm*

Three right-handed subjects (2 females) participated in this pilot experiment. The average age of the subjects was 25 years (range 21-29 years) and none had a history of respiratory dysfunction or asthma.

Subjects respiration was measured with a respiratory monitoring belt fitted around the subject's chest. Subjects were instructed to take a deep breath when cued

by a tone. A brief (1s) cueing tone was presented every 30 seconds and for a total of 15 cycles.

2.4.4.2 Image acquisition and data analysis

Both functional and structural MRI images were taken on a Siemens/Varian 3T system fitted with a birdcage head coil. Subjects were immobilised using cushions to reduce head motion. For the functional data series, 225 T2*-weighted images depicting BOLD contrast (Ogawa et al. 1990) were acquired over a scan duration of 450s. The scans had an in-plane resolution of 3mm at each of 21 axial 5mm thick slices covering the entire cerebrum and cerebellum. The acquisition parameters were TR = 2s; TE = 30ms, field of view = 224 x 224; matrix = 64 x 64 and flip angle of 75°.

Prior to data analysis, the first 3 volumes were deleted. Analysis was carried out using FEAT4, the FMRIB Expert Analysis Tool (Smith et al. 2004). The following pre-statistics processing was applied: motion correction using MCFLIRT (Jenkinson and Smith 2001); spatial smoothing using a Gaussian kernel of FWHM 5mm; mean-based intensity normalization of all volumes by the same factor; non-linear highpass temporal filtering (Gaussian-weighted LSF straight line fitting, sigma=36.0s). Statistical analysis was carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich et al. 2001). Z (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z > 2.3$ and a cluster significance threshold of $P = 0.01$ (Forman et al. 1995; Friston et al. 1994; Worsley et al. 1992). This particular threshold was chosen because pilot studies suggested that this level constitutes the best compromise between sensitivity to

activation and the avoidance of false positives. Registration to high resolution images was carried out using FLIRT.

2.4.4.3 Results

The subjects respiratory cycles remained fairly constant throughout the experiment at approximately 7 seconds per cycle. Subjects reliably produced a deep inhalation following the brief auditory cue. The deep inhalation resulted in a chest expansion by a factor of approximately 4 compared to normal breathing. An example recording of one subject's respiration pattern during the experiment as recorded by the respiratory monitoring belt is shown in Figure 2.13.

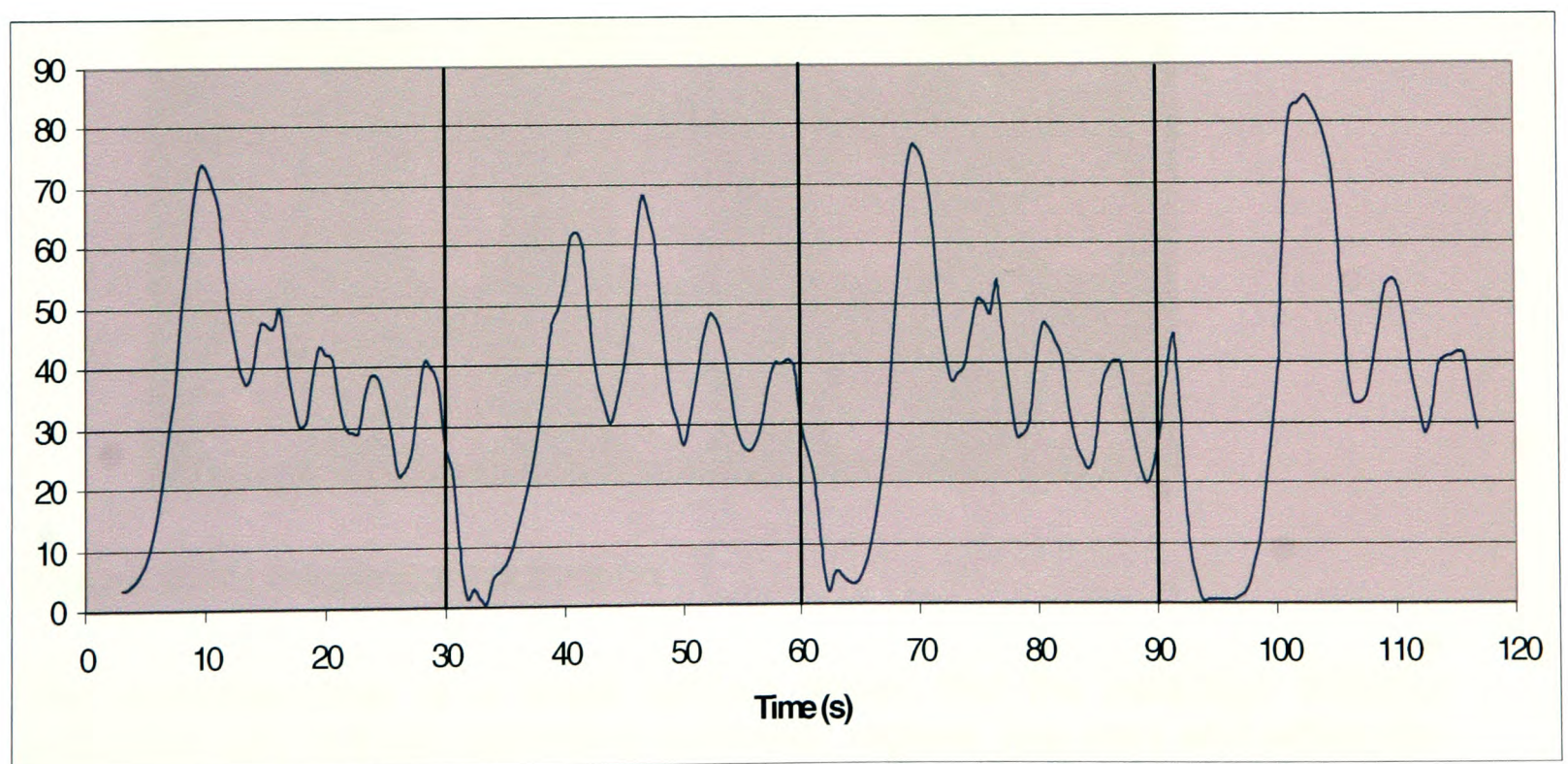


Figure 2.13: Example respiratory recording.

The blue curve depicts the respiratory pattern of the subject and the black lines mark the onset of the auditory cue. A clear alteration in the respiratory pattern following the auditory cue can be observed.

No significant activation could be observed in any of the subjects in olfactory areas throughout the orbitofrontal cortex and in the piriform cortex. However, a substantial change in fMRI signal strength, which was in phase with the subject's respiratory cycle, was observed in the brainstem, the cerebellum and the lateral ventricles (Figure 2.14). This effect was particularly pronounced in areas bordering the ventricles, suggesting that partial volume effects are responsible for this pattern of activation. Furthermore, the temporal changes in MR signal are in almost perfect synchronicity with the respiratory pattern (Figure 2.15).

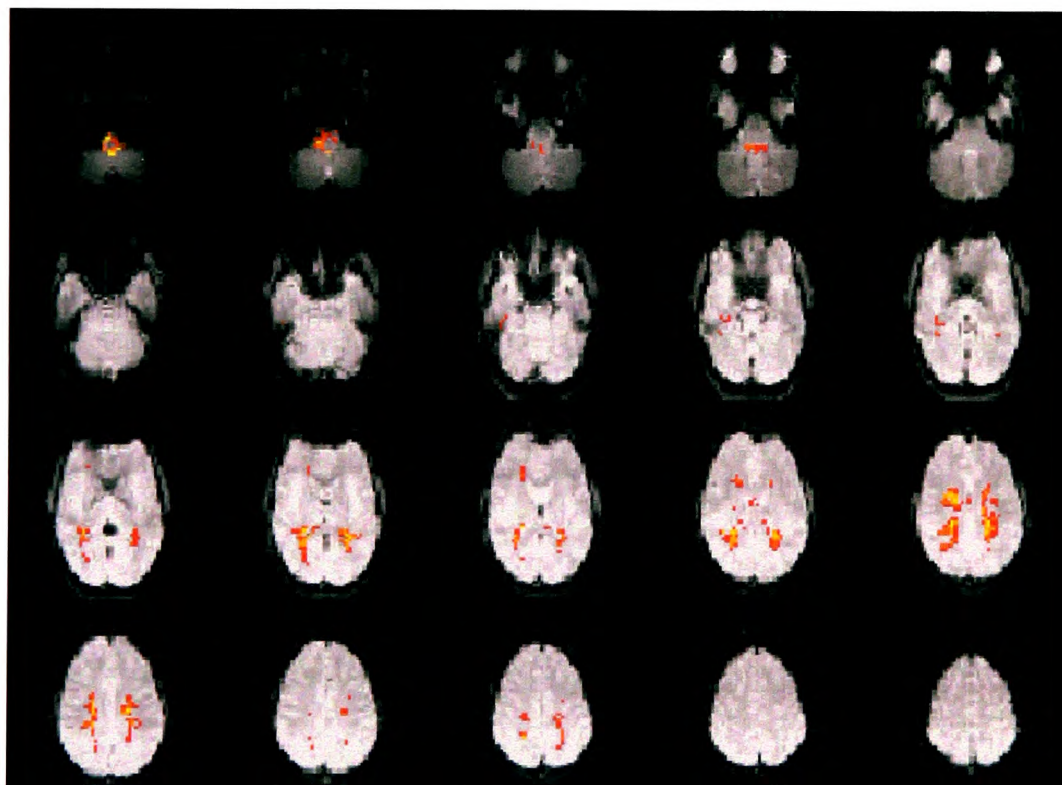


Figure 2.14: Respiratory activation.

The activation map of a single subject shows that the paradigm induced activation only in brain stem and cerebellar regions (top row) and within the lateral ventricles. This effect is most likely to be caused by stimulus correlated motion, rather than neural activity.

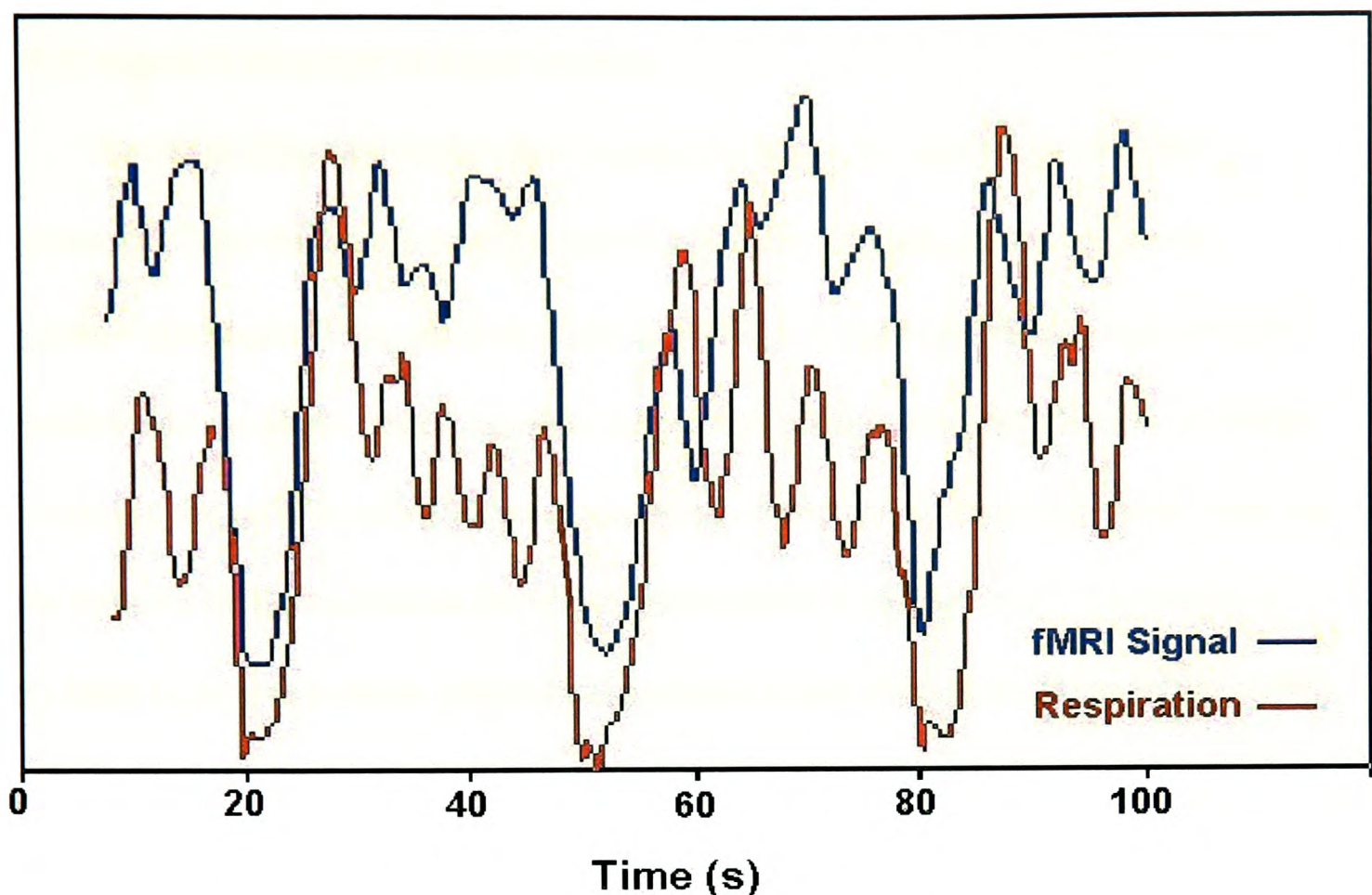


Figure 2.15: Respiratory induced fMRI signal changes.

Example data from a single voxel within the brainstem showing the correlation between the time course of the fMRI signal and the subject's respiration (both in arbitrary units). The red curve shows the recorded respiration and the blue curve the recorded fMRI signal.

2.4.4.4 *Conclusions*

A significant correlation between subjects respiration and the fMRI signal was observed, but not in areas associated with olfactory perception. This effect was particularly strong along tissue boundaries, suggesting that these activations are due to partial volume effects. Additionally, a small cluster of activation was detected within the cerebellum consistent with previous studies on sniffing (Sobel et al. 1998a; Sobel et al. 1998b). However, the observation that both MR signal and respiration are fluctuating synchronously suggests that the observed activation is due to stimulus

correlated motion, because neural activity should result in a temporal lag of the BOLD signal in the order of a few seconds.

Because olfactory stimulation essentially has to be phase locked to the respiratory cycle, this could result in artefactual activation during an olfactory activation paradigm. This problem arises especially in regions close to high-contrast boundaries in the brain image, because movement related intensity changes are large. As a result, the motion related intensity changes could not be distinguishable from the ones induced by the activation paradigm, particularly if the olfactory stimulation is very brief ($<3s$). As a result, olfactory stimulation times in the studies presented in this thesis were prolonged to 6-8s. Whilst this dramatically decreased the influence of motion artefacts, it also may have led to a reduced height of the BOLD response due to habituation effects. As mentioned in the previous chapter, the BOLD response to continuous olfactory stimulation in piriform cortex is characterised by a sharp increase that lasts for about 10 seconds, followed by a rapid decrease to levels below baseline (Poellinger et al. 2001). Olfactory stimulation in the order of 6-8 seconds should not be negatively affected by this. However, as demonstrated by Sobel and colleagues (2000) the height of the BOLD response over the course of an experiment is continuously reduced due to habituation effects. Clearly, increased stimulus durations will make this effect more pronounced. Nevertheless, the results of pilot experiments suggested that the effects of habituation are small compared to confounds produced by motion.

Clearly, finding an alternative means of delivering olfactory stimuli would be desirable, where olfactory stimulation is not phase-locked to respiration. One way around this problem is to employ nasal prongs, which deliver the odour directly onto the nasal epithelium. If subjects are taught a special breathing technique called

velopharyngeal closure (Kobal 1985) where they are asked to breathe through their mouth whilst closing the passage to the sinuses, stimuli can be delivered non-synchronously to breathing, thus eliminating stimulus-correlated artefacts. However, this technique is not very comfortable for the subject and additionally is a very artificial way of perceiving an odour. It could also be argued that active inspiration directs attention to the olfactory modality, which in turn would induce larger BOLD signal changes. Additionally, because the observed change in the MR signal after an inspiration 4 times larger than normal breathing did not result in any detectable activation in olfactory brain regions, respiratory correlated motion in normally breathing subjects does not appear to affect imaging this region. Taken together, these observations meant that all subsequent experiments were carried out with freely breathing subjects.

3 Sensitivity encoded spiral imaging

The discovery of alternative methods to echo-planar imaging (EPI) has made it possible to acquire images in a faster time frame. Particularly spiral imaging using sensitivity encoding (SENSE) is a promising novel technique for fMRI, since faster acquisition time should lead to reduced image artefacts, which is exceptionally useful for investigations of orbitofrontal cortex function. In the present chapter, the SENSE technique was applied to functional MRI with BOLD contrast in a taste paradigm and compared to a conventional spiral acquisition. Results confirmed that the SENSE acquisition lead to increased image quality in the OFC.

3.1 INTRODUCTION

Spiral readout techniques are frequently used in functional MRI (fMRI) of the brain based on blood-oxygen-level-dependent (BOLD) contrast (Noll et al. 1995) due to their superior motion and flow properties compared with echo-planar imaging (Glover and Lee 1995; Nishimura et al. 1995). The main feature that distinguishes spiral imaging from EPI is the way that k-space is sampled (see (Block and Frahm 2005) for a recent review). During EPI, k-space is sampled in a back-and-forth trajectory following a single excitation pulse and thus requires rapidly switching gradients. Additionally, data collected during transition from one line of k-space to the next is not used for image reconstruction. The speed with which a volume of brain

can be acquired is hence limited by the strength of the gradient and the slew rate with which the gradient can be changed. Accordingly, improvements in gradient performance have been areas of much hardware development in the past, resulting in greatly reduced scan time for EPI. However, the technical and physiological limits of speed at which k-space is traversed have almost been reached.

In contrast, spiral imaging techniques sample k-space in an Archimedean spiral path, i.e. with equidistant revolutions that typically begin at the centre of k-space (Glover 1999). This requires sinusoidal changes in the gradient that are much less taxing on the gradient system, resulting in faster sampling of k-space. Furthermore, all the points sampled in k-space are used for image reconstruction, which increases efficiency. Together, these result in much faster image acquisition for spiral imaging compared to EPI.

In spiral imaging, complete images are usually acquired in a single shot after spin excitation to provide high time efficiency and to avoid sensitivity to inter-shot motion. However, depending on the required spatial resolution and the available gradient system this still leads to relatively long acquisition windows in the range of 20-100 ms. During this period of time, off-resonance phase errors evolve due to susceptibility gradients or chemical shift differences. Additional blurring results from signal loss caused by transverse (T_2^*) relaxation during the readout period (Farzaneh et al. 1990). The conjugate phase reconstruction (CPR) method (Man et al. 1997) partly enables correction of off-resonance errors even in the case of non-Cartesian trajectories, i.e. for spiral imaging methods. However, CPR assumes a certain smoothness of the spatial off-resonance distribution and fails in cases where strong susceptibility gradients are present, such as the orbitofrontal cortex (OFC). Furthermore, it cannot account for signal loss due to through-plane dephasing or T_2^*

decay. One way to address both types of dephasing is to shorten the acquisition window using multiple spiral interleaves. However, motion sensitivity has to be dealt with by using some kind of navigation (Glover and Lai 1998), and more excitations are needed to complete an image, leading to reduced temporal resolution.

A novel approach of shortening the acquisition time has recently been introduced which requires the use of multiple receiver coils (Sodickson and Manning 1997). These parallel acquisition techniques using receiver coil arrays have proven capable of shortening readout trains in Cartesian single-shot imaging, thereby reducing artefacts (Bammer et al. 2001) and improving spatial resolution (Griswold et al. 1999) without sacrificing signal-to-noise ratio. The image acquisition time is reduced by exploiting spatial information inherent in the geometry of a receiver coil array to substitute for some of the phase encoding usually produced by magnetic field gradients. The technique of sensitivity encoding (SENSE) (Pruessmann et al. 1999) is based on the fact that the induced voltage in a receiver coil by a given signal source varies considerably according to its relative position. Therefore, knowledge of the spatial receiver sensitivity can be used to calculate the spatial origins of the detected MR signals and may be utilized for image generation. Unlike position in k -space, sensitivity is a property of the receiver and is independent from the state of the object under examination. By using distinct receivers in parallel, scan time can be reduced without having to travel faster in k -space. Therefore, scan time is reduced at preserved spatial resolution. The factor by which the number of k -space samples is reduced is referred to as the *reduction factor* R . The total acquisition time per image can theoretically be reduced by a factor equal to the number of independent surface coils. In practice, a reduction factor of $R = 2$ is generally used, largely because higher reduction factors are not trivial to achieve, even with multiple receiver coils. The

reduction has the undesirable consequence of decreasing the signal-to-noise ratio (SNR) by a factor equalling $\sqrt{R}$. Recently, an efficient algorithm for iterative reconstruction of data from arbitrary trajectories has been described (Pruessmann et al. 2001) that particularly makes spiral SENSE feasible for BOLD imaging experiments.

In the present work, SENSE was used in a gustatory activation paradigm with sweet and salty liquid stimuli because it enables single-shot spiral imaging with a reduced readout window. The benefits with respect to off-resonance artefacts and spatial resolution were investigated in comparison with a conventional spiral acquisition using the same stimulation paradigm. Taste stimulation was specifically chosen as the activation paradigm, because gustation is known to elicit BOLD changes in the OFC (Small et al. 2003; Zald et al. 2002), an area that is particularly prone to susceptibility artefacts. Additionally, the particular stimuli used in the present study have previously been shown to elicit responses in insula cortex, amygdala and OFC (O'Doherty et al. 2001). The main aim of the study was to investigate whether the SENSE acquisition would reduce susceptibility artefacts in OFC and thus present a feasible alternative method to EPI for investigations concerning OFC function, in particular the chemical senses.

3.2 METHODS

3.2.1 Sensitivity Encoded Spiral Imaging

The experiments were based on a common protocol for single-shot spiral BOLD fMRI. Using a field-of-view (FOV) of 240 mm and a matrix size of 80, the trajectory was designed using the method described by Börnert and colleagues (Börnert et al. 1999). The readout duration of the conventional spiral trajectory was 36 ms using a maximum gradient strength of 18.2 mT/m and a maximum slew rate of 99.4 mT/m/ms. For SENSE imaging a reduction factor of $R = 2$ was applied by reducing the sampling density in the radial direction while keeping it constant along the trajectory. This enabled shortening of the acquisition window to 18 ms without altering the demands on the gradient peak values. The whole brain was covered with 24 contiguous axial slices of 5 mm thickness, acquired every 2.5 s in an interleaved manner and using equal temporal spacing. Spin excitation was performed using a water-selective spectral-spatial pulse with a flip angle of 90° . Images were acquired with an echo time (TE) of 5 ms, as well as 35 ms in the functional studies.

For registration into standard space, a high-resolution, whole-brain T1 weighted morphological scan (inversion-recovery fast gradient echo, 1.0mm slice thickness, 1.0 mm $\times$ 1.0mm in-plane resolution) was acquired after the functional paradigm.

The experiments were carried out at 1.5 T on a Gyroscan Intera (Philips Medical Systems, Best, The Netherlands) MR imaging system, equipped with a Powertrak 6000 gradient system and based at the Institute for Biomedical Engineering, Swiss Federal Institute of Technology, Zürich, Switzerland. A receiver coil array with six rectangular elements of 10 cm $\times$ 20 cm was used for the SENSE as

well as the conventional spiral protocol. The coils were arranged around the head without overlapping adjacent elements, as depicted in Figure 3.1. Tight padding with foam wedges was used to restrict subject's head movement. Five healthy volunteers (2 females, mean age 26 years) were scanned after informed consent was obtained.

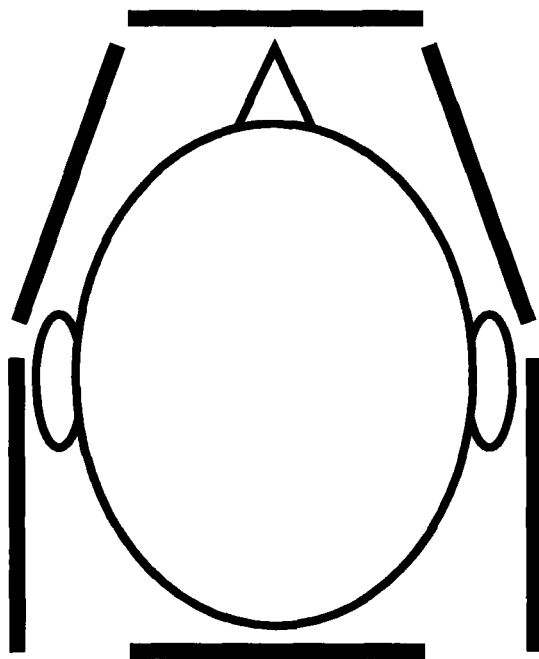


Figure 3.1: Setup of the coil array.

The six rectangular elements (10 cm $\times$ 20 cm) were arranged around the head, as indicated by the black bars. The larger dimension of the coils was aligned with the feet-head direction and adjacent elements were not overlapping.

Reference images for sensitivity calibration were obtained in vivo with the coil array and the body RF coil acting as receivers in subsequent scans (a volume head coil could equally be used in high-field scanners that do not possess a body RF coil). The reference images were acquired with the full k-space spiral protocol described above, as well as with a gradient-spoiled Cartesian gradient-echo sequence for comparison. The latter sequence was performed with the same FOV, slice thickness and slice

positions as in the spiral sequence, but with matrix = 128, TR = 7 ms, TE = 3.8 ms, and flip angle = 30°. From the reference images the coil sensitivity maps were generated as described by Pruessmann and colleagues (Pruessmann et al. 1999). It was found that both reference protocols served equally well to generate reliable sensitivity maps. Note that the body coil images are only necessary to perform intensity correction of the images after SENSE reconstruction.

Spiral reconstruction was carried out using the iterative gridding approach (Pruessmann et al. 2001) and applying both k-space density and coil intensity correction. Based on the conjugate gradient method, the algorithm calculates a progression of images that converges towards exact reconstruction. In practice this is done by flipping the image data back and forth between image domain and the k-space domain, using forward and reverse FFT and gridding operations. Reconstructing the SENSE data in the present work required four iterations for each image. Note that the first iteration step is equivalent to standard gridding reconstruction with optimum-SNR coil combination. Therefore, to reconstruct the fully Fourier-encoded data the same procedure was used and simply stopped after the first loop.

3.2.2 fMRI Paradigm

For functional imaging, a block design paradigm was chosen to compare taste stimulation detected by the two spiral techniques. The order of the two acquisitions with and without SENSE was randomised. Stimulation was performed using sweet and salty liquids in a random order to avoid habituation effects. An additional tasteless liquid was used as a control condition and was being administered after each

taste stimulation to enable elimination of activation associated with tongue movement and swallowing. The taste stimuli were mixed using double de-ionised water to yield a sweet (1 M glucose), a salty (0.1 M NaCl), and a tasteless (25 mM KCl and 2.5 mM NaHCO₃) liquid. The choice of this particular ionic composition for both the taste stimuli the tasteless control solution was based on previous reports by Rolls and colleagues (O'Doherty et al. 2001; Rolls 2005). Additionally, using water as a control stimulus is not feasible since it is known to activate taste regions in the OFC (de Araujo et al. 2003b). The three liquids were each supplied to the volunteers' mouth by means of a flexible Teflon tubing (inner $\varnothing = 1.0$ mm), a manually operated syringe, and a bottle containing the respective fluid. For each trial, 0.75 ml of each of the different liquids was given, injected over the period of 1s. The subjects were asked to taste the liquid by moving it around their tongue for 10 s until they were visually cued to swallow. They were particularly requested to minimise any head movement during this task. Each of the tasting periods (36 in total) was followed by an inter-stimulus interval of random duration between 5 and 12.5 s, and each 'taste' trial was always followed by a 'tasteless' trial.

3.2.3 Data Analysis

For evaluation of the fMRI data three statistical measures were used, as previously proposed (Glover and Lai 1998). From the magnitude image time series, the number of activated voxels (activation volume), the signal-to-noise-ratio (SNR), and the signal-to-fluctuation-noise-ratio (SFNR) were determined.

fMRI analysis was carried out using FEAT, the fMRI Expert Analysis Tool (Smith et al. 2004). The following pre-statistics processing was applied: motion

correction using MCFlirt (Jenkinson and Smith 2001), spatial smoothing using a Gaussian kernel (5 mm wide), and nonlinear high-pass temporal filtering (Gaussian-weighted, least-squares, straight line fitting with the kernel width $\sigma = 1.5 \times$ duration of one cycle). Statistical analysis was carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich et al. 2001). Z (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z > 2.3$ for the taste paradigm and a cluster significance threshold of $P = 0.01$ (Forman et al. 1995; Friston et al. 1994; Worsley et al. 1992). For the analysis of the taste data, sweet and salty periods were merged to form the 'taste' event, whereas the tasteless cycles were used to form a control condition.

SNR and SFNR were calculated as described by Glover and Lai (1998), using the full 3D data set and all cycles of the paradigm. The data were pre-processed with motion correction and high-pass temporal filtering as described above. Activated voxels were excluded from this evaluation. For calculating the SNR, a square region-of-interest (ROI) of 6×6 voxels was moved voxel by voxel over the generated signal and noise images, independently for each slice. ROIs containing less than 70% of valid voxels (not outside head and not activated) were excluded, as were those exhibiting extremely high motion- or flow-related noise values (e.g. the sagittal sinus). Mean values of SNR and SFNR were calculated over all accepted voxels in the imaging volume.

3.3 RESULTS

Single-shot spiral images with a short TE of 5 ms were acquired prior to the fMRI acquisition in order to investigate image quality without the effect of extensive signal dephasing. Figure 5.2 shows results at a selected slice location to demonstrate the effects of a reduced readout window. With conventional spiral imaging (Figure 3.2A) there is a strong off-resonance artefact in the orbitofrontal region (top, bold arrow). This is due to the large susceptibility gradients caused by the neighbouring air-filled sinuses that cause geometric distortions, which in turn lead to a dislocated signal being accumulated in the bright ring. This effect is strongly reduced with spiral SENSE imaging (Fig. 5.2B). The reduced blurring also visibly improves the delineation of edges (bottom arrow) and of the fat signal (left arrow). The expected increase in resolution due to reduced signal dephasing during the shorter readout is observable when small structures (such as the ventricles) are examined. Also associated with the improved resolution is a slightly higher contrast.

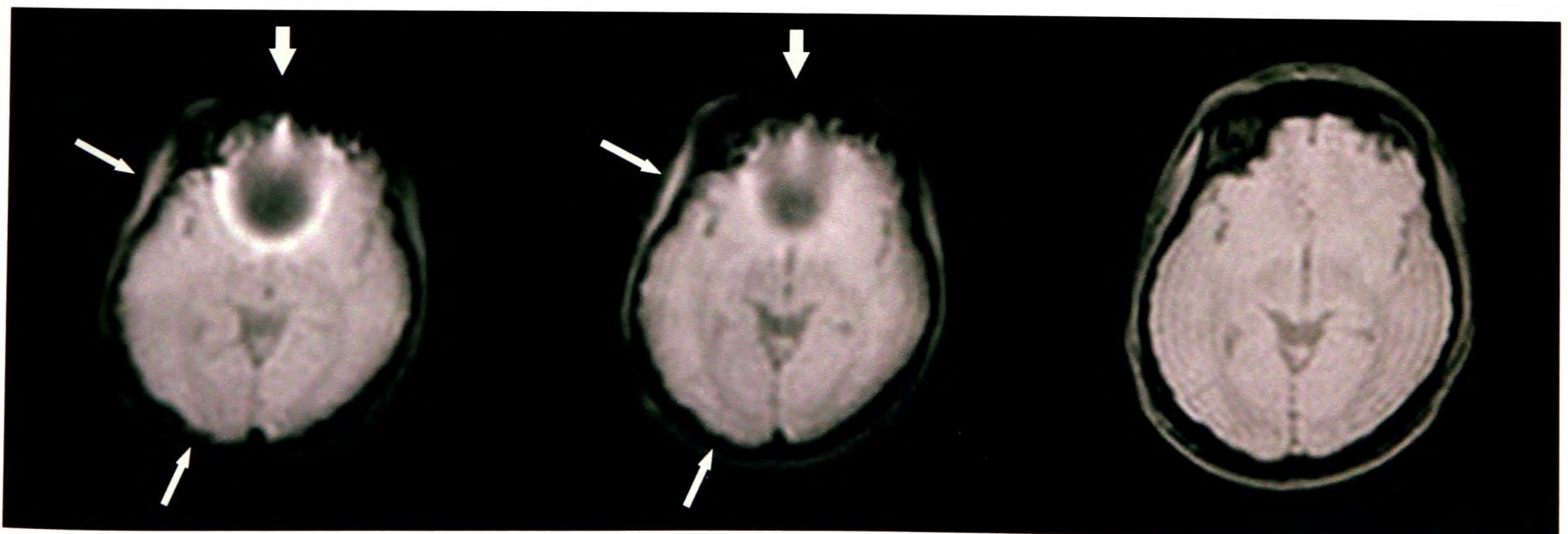


Figure 3.2: Single-shot spiral imaging at TE = 5 ms.

(A) Conventional spiral with a readout duration of 36 ms. (B) Spiral SENSE with 18 ms readout. (C) Conventional gradient-echo image with the same nominal resolution and without k-space filtering for reference.

The loss of SNR in Figure 3.2B vs. Fig 3.2A (see below) is hardly observable, largely due the relatively high base SNR. As a reference for anatomy and image quality Fig. 3.2C shows a conventional gradient-echo image acquired with the same matrix size. All images in Fig. 3.2 were Fourier-interpolated to a matrix size of 240 using zero filling, which enables the comparison of the effect of k-space filtering for the different acquisition schemes. The distinct Gibbs ringing in Fig. 3.2C is strongly reduced in the spiral images due to filtering associated with T2* decay. However, the spiral SENSE image with the shorter readout duration, and hence less filtering, still exhibits slight ringing.

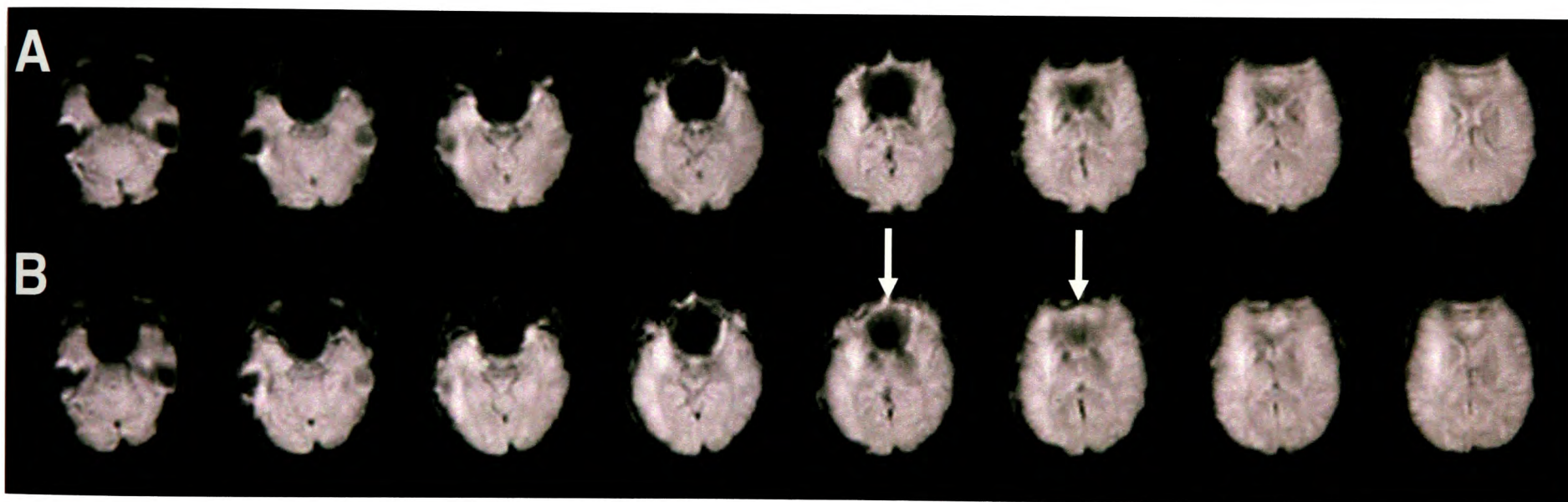


Figure 3.3: Single-shot spiral imaging at TE = 35 ms.

(A) Conventional spiral, and (B) SENSE acquisition. Eight adjacent slices, out of 24 slices, were selected after registering the two data sets to an intermediate position. Note the reduced signal dropout in OFC for the SENSE acquisition (white arrows).

Images sensitised for BOLD fMRI with the longer TE of 35 ms are shown in Figure 3.3. For optimal comparison, the conventional spiral and SENSE spiral three-dimensional data sets were registered to an intermediate position using translation and rotation. Thus, the slight loss in resolution associated with the interpolation affects

both image sets equally. The conventional spiral images (Fig. 3.3A) show predominantly signal loss rather than strong blurring artefacts in the orbitofrontal region, which is in contrast to Fig. 3.2A. This is because most of the signal that blurred heavily during the spiral readout had already been dephased prior to actual functional acquisition. Nevertheless, the SENSE images in Fig. 3.3B again show a considerable improvement in image quality, although the benefit is not as large as with a short TE.

Table 1 summarizes the statistical analysis of the fMRI experiments. SNR and SFNR values on the order of 100 were determined for all data sets. The SNR ratio of SENSE vs. conventional spiral images, averaged over all subjects, is 0.79. Not surprisingly, this value is slightly higher than the $1/\sqrt{2} = 0.71$ ratio that would be expected by considering the shortened acquisition time alone. This is because additional signal loss occurs during the second half of the conventional readout, which does not occur in the SENSE acquisition. Furthermore, this suggests that SENSE-specific noise was negligible in the present setup. The respective SFNR ratio of 0.83 is slightly higher than the SNR ratio, indicating a reduced sensitivity of the faster SENSE acquisition to fluctuations on the time scale of the readout duration.

	SNR	SFNR	Activation volume
Subject 1			
<i>Conventional</i>	<i>138</i>	<i>115</i>	<i>2273</i>
<i>SENSE</i>	<i>94</i>	<i>88</i>	<i>5039</i>
<i>Ratio</i>	<i>0.68</i>	<i>0.77</i>	<i>2.22</i>
Subject 2			
<i>Conventional</i>	<i>124</i>	<i>112</i>	<i>2830</i>
<i>SENSE</i>	<i>96</i>	<i>85</i>	<i>472</i>
<i>Ratio</i>	<i>0.77</i>	<i>0.76</i>	<i>0.17</i>
Subject 3			
<i>Conventional</i>	<i>141</i>	<i>111</i>	<i>765</i>
<i>SENSE</i>	<i>123</i>	<i>106</i>	<i>377</i>
<i>Ratio</i>	<i>0.87</i>	<i>0.95</i>	<i>0.49</i>
Subject 4			
<i>Conventional</i>	<i>125</i>	<i>110</i>	<i>1232</i>
<i>SENSE</i>	<i>100</i>	<i>94</i>	<i>346</i>
<i>Ratio</i>	<i>0.80</i>	<i>0.85</i>	<i>0.28</i>
Subject 5			
<i>Conventional</i>	<i>148</i>	<i>136</i>	<i>527</i>
<i>SENSE</i>	<i>121</i>	<i>110</i>	<i>796</i>
<i>Ratio</i>	<i>0.82</i>	<i>0.81</i>	<i>1.51</i>
Average Ratio	<i>0.79 ± 0.07</i>	<i>0.83 ± 0.08</i>	<i>0.93 ± 0.89</i>

Table 3.1: Analysis of SNR, SFNR and activation volume.

Only non-activated voxels of the entire brain volume were included in both SNR and SFNR calculations and are shown individually for each subject. Activation volume was calculated by the sum of activated voxels (size = 3 mm × 3 mm × 5 mm) after cluster thresholding with $Z > 2.3$ and $p < 0.01$ (corrected for multiple comparisons).

The activation in the fMRI experiments was quantified by the number of activated voxels, yielding the activation volumes listed in Table 3.1. These volumes are derived from the results of the 'taste' event and include all activations associated with that task. In the contrast 'taste versus tasteless', hardly any significant activation was observed. This may be related to the problem that, as reported by some of the

subjects, the 'tasteless' liquid had a slightly salty/bitter taste (see Chapter 5 for a more thorough discussion of these issues).

The normalized activation volume ratio (averaged over all subjects) of 0.93 suggests that SENSE and the conventional acquisition performed approximately similar, albeit with a very large standard deviation of 0.89. A comparison of the individual activation volumes reveals diverging results throughout the subjects, with ratios ranging from 0.17 to 2.22. This broad range of ratios in the activation volume indicates difficulties in reproducing the activation with this paradigm. However, higher volumes were consistently observed during the first experiment, irrespective of acquisition method. This strongly suggests habituation affecting the cerebral response to the gustatory stimuli. Some of this variability may be ascribable to signal perturbation resulting from residual motion following tongue movement and swallowing, although motion correction results did not suggest significant effects. No correlation was found between the ratios of activation volume and those of SNR and SFNR.

An example of a direct comparison of the activation pattern and location from the two subsequent scans showing good reproducibility is found in subject 5. Figure 3.4 shows a slice from this subject, in whom reduced signal loss enabled the recovery of a robust taste activation. The raw images on the left show slightly more signal in the medial aspects of the orbitofrontal cortex for the SENSE acquisition (Fig. 3.4C) than for the conventional spiral image (Fig. 3.4A). Indeed, the average signal of brain voxels in this region was 20% higher with SENSE than with the conventional spiral. Accordingly, activation in this region at a clustering threshold of $z = 2.3$ is only visible in the SENSE case (Fig. 3.4D).

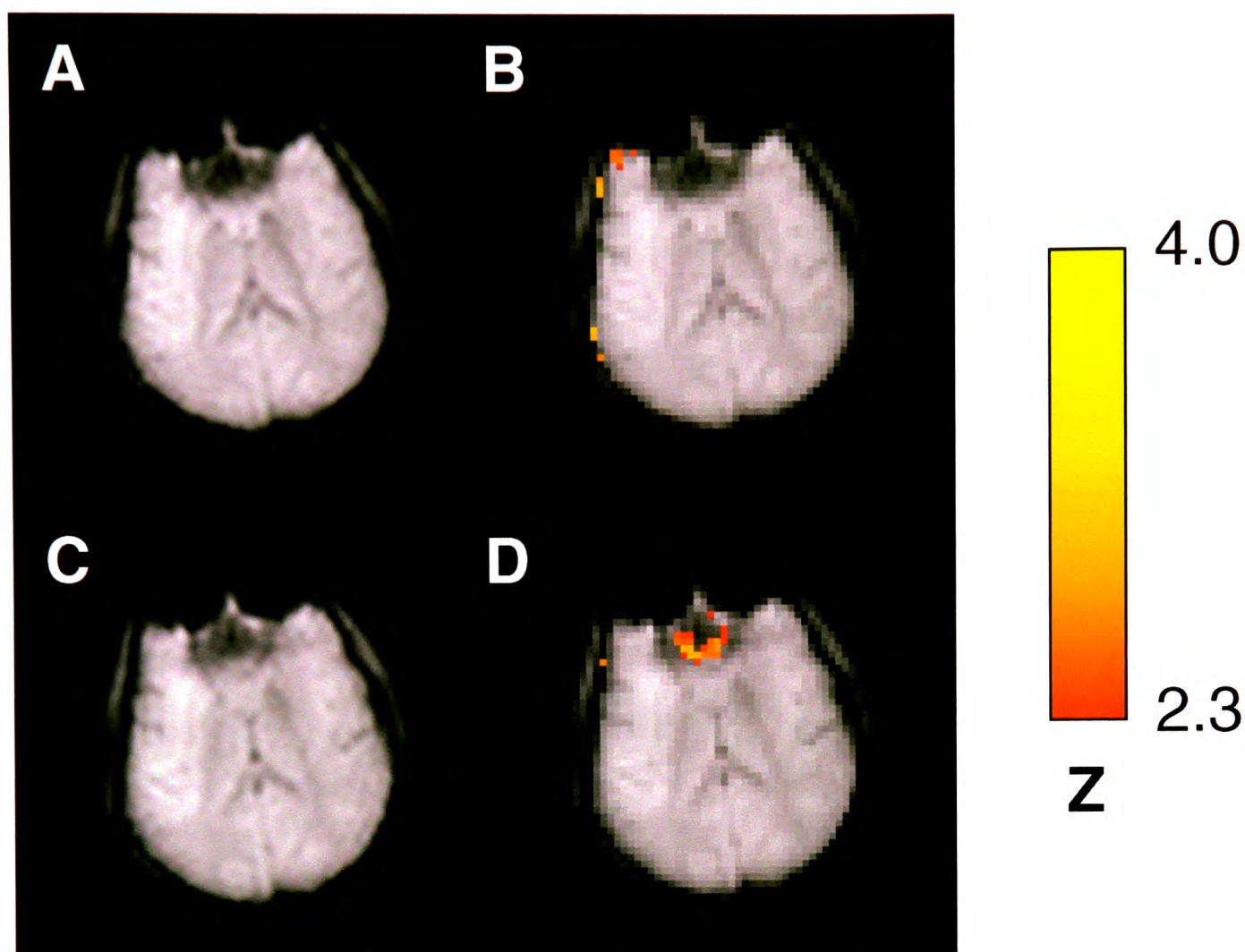


Figure 3.4: Activation in response to taste stimulation.

A slice location from subject 5 is shown with the raw images from (A) conventional and (C) SENSE spiral imaging. Note the slightly improved signal intensity in the orbitofrontal region in the SENSE image. B and D: the activated voxels are overlaid onto the raw images, using the colour bar on the right to indicate the respective Z values after cluster thresholding with $Z > 2.3$ and $p < 0.01$ (corrected for multiple comparisons). With (D) the SENSE acquisition, activation in medial orbitofrontal cortex was detected, which is not present in (B) the conventional case. Activated voxels at the extreme border of the brain are most likely caused by stimulus-correlated motion.

To further characterise the results of the taste study, a fixed effect group analysis was performed for the two image acquisition techniques separately. The full group analysis including all subjects showed clear differences between the conventional and SENSE spiral acquisitions, with zero activation volume for the conventional acquisition versus 1518 voxels for the SENSE acquisition. However, the previous single-subject analysis suggested that Subject 3, who showed little response

in the conventional scan and an unusual pattern of activation in the SENSE scan, might have biased this difference. To determine whether the result was robust to this outlying subject, a further analysis, from which Subject 3 was excluded, was performed. Figure 3.5 shows the latter group analysis result, demonstrating activations in insula cortex and adjoining frontal operculum as well as in the medial orbitofrontal cortex, corresponding to known primary and secondary taste areas (O'Doherty et al. 2001; Small et al. 2003; Zald et al. 2002). Additionally, activation in the left anterior insula cortex and frontal operculum could only be detected in the SENSE acquisition.

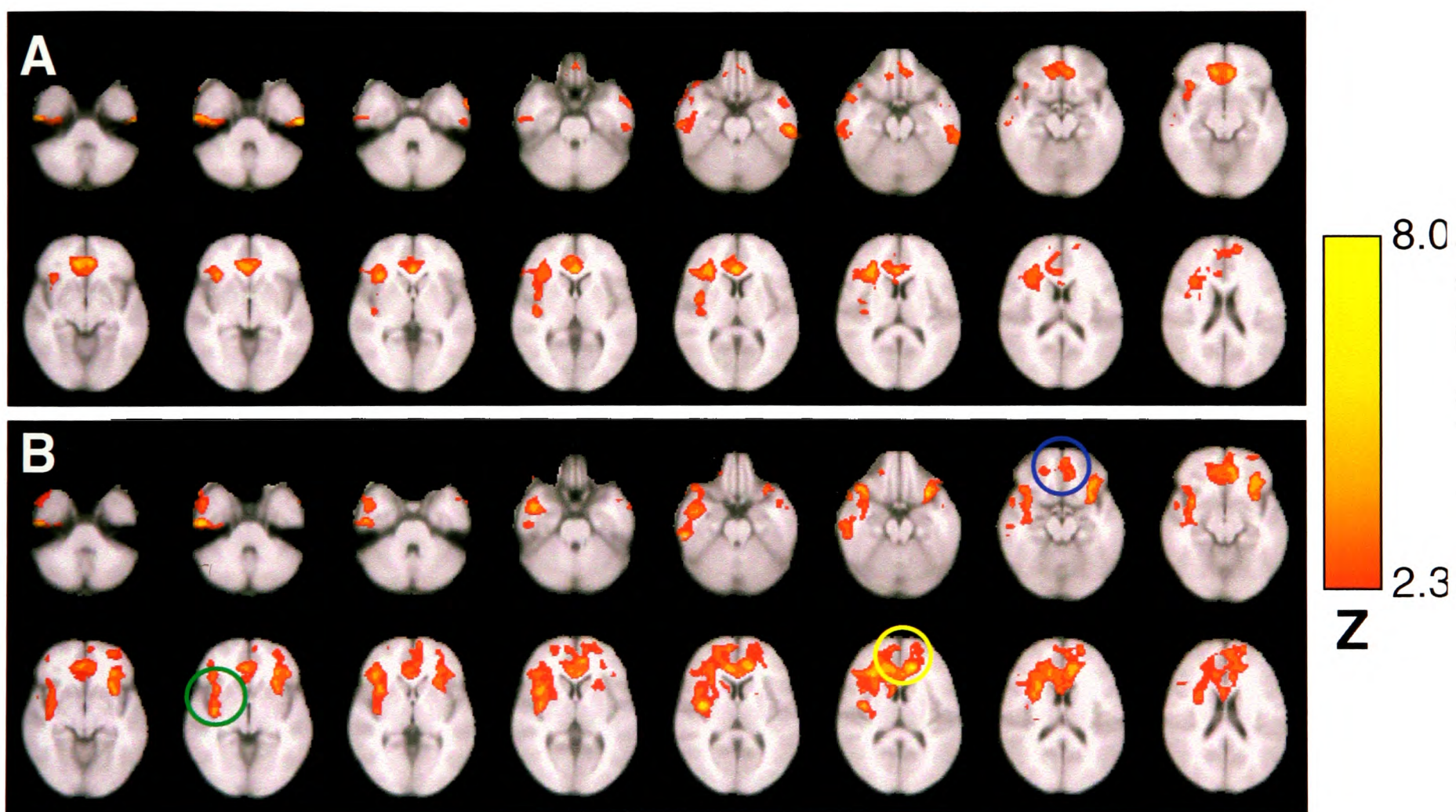


Figure 3.5: Fixed-effect group results.

The results of the group activation for (A) conventional spiral imaging and (B) SENSE acquisition are depicted, rendered onto the MNI-305 standard brain. Both results show taste activation in medial OFC (blue), insula cortex and adjoining frontal operculum (green) and anterior cingulate (yellow). However, with the SENSE acquisition a greater extent of activation is detected than with conventional spiral imaging. The colour bar indicates the respective Z values after cluster thresholding with $Z > 2.3$ and $p < 0.01$ (corrected for multiple comparisons) for the fixed-effect group analysis ($n=4$). Note that the right side of the brain is depicted on the left side of the image.

Activation was additionally detected in the anterior cingulate, which is most likely to be task-related rather than specific to the gustatory stimulation. Activation in insula cortex was found for both analyses to be lateralised to the right hemisphere, and was predominantly in the anterior part of the insula cortex. The SENSE data (Fig. 3.5B) exhibits generally a larger volume of activation (3071 voxels) in all the above regions compared to the conventional scan (1704 voxels). All of these areas correspond to regions of the brain that suffer from distortions associated with either the frontal or temporal lobe air/tissue interfaces. In certain areas (e.g., the inferior orbitofrontal parts in slices 4 – 7), the conventional spiral gave better results (Fig. 3.5A). Overall, and despite the problem of limited reproducibility mentioned above, the group analysis also demonstrated the potential of the SENSE approach to enhance BOLD imaging of frontal areas close to problematic air/tissue interfaces, and implies a greater consistency in the spatial location of the activations detected with SENSE.

3.4 DISCUSSION

This study demonstrated that image acquisition using SENSE enables shortening of the readout duration in single-shot spiral imaging. As a result, off-resonance blurring and signal loss can be reduced and resolution can be improved. Therefore, spiral SENSE has the potential to improve the detection of BOLD fMRI activation in brain regions affected by signal dephasing and artefacts induced by susceptibility gradients, namely the OFC.

With SENSE, the time efficiency of spiral data acquisition was increased to a level not attainable with gradient encoding alone. In fact, for similar results the conventional approach would require a peak gradient strength of at least 36 mT/m at a peak slew rate in the order of 400 mT/m/ms, which is beyond current technical and physiological limitations. Moreover, the SENSE concept entails a reduction in the gradient duty cycle, which on many scanners still forms a serious restriction to lengthy protocols such as those used in most fMRI studies.

As a prerequisite, parallel imaging techniques use receiver coil arrays (six in the present study). Consequently, the images obtained exhibit inhomogeneous noise levels, just as in conventional surface coil imaging with intensity correction. Typically, with a well-designed coil array the SNR near the object's surface border is increased by factors of 2-4 as compared with a birdcage head coil, whereas in the object centre it is slightly reduced by 10 –20%. This leads to a variation in SNR within a single axial slice by a factor of as much as 5. Furthermore, the SNR variation between the central and outer slices can also be up to a factor of 3. In addition, SENSE reconstruction may enhance SNR inhomogeneity by geometry-related noise amplification, especially at reduction factors larger than two (Pruessmann et al. 1999). Therefore, when performing fMRI experiments with parallel imaging techniques, one

has to be aware of the fact that equally strong activation may be detected with different statistical significance depending on the respective local SNR.

In the present study an iterative algorithm was used to reconstruct spiral SENSE images, which is the most efficient approach known to date for obtaining alias-free images from undersampled, non-Cartesian data (Pruessmann et al. 2001). Each iteration step takes approximately the same amount of CPU time per coil as conventional gridding reconstruction. The number of iterations required for proper convergence depends on the conditioning of the reconstruction problem. With the coil setup used here, and two-fold SENSE reduction, four iterations were sufficient in all cases. Hence, relative to common spiral imaging with a single coil the use of SENSE with six coils caused a 24-fold increase in processing time. Consequently the processing of each fMRI data set took several hours. This increased computational demand for SENSE image reconstruction, together with the fact that practical implementation of the technique at a standard MRI scanner is not trivial to attain and requires specialised personnel, may well hinder a more widely spread use of the technique. Nevertheless, continued increases in CPU performance and the use of parallel reconstruction are likely to reduce reconstruction times to a convenient range in the near future. Additionally, SENSE imaging might prove particularly useful for ultra high-field (≥ 4 T) fMRI investigations, since the extent of the susceptibility artefacts increases with field strength.

In agreement with earlier studies (Glover and Lai 1998), the activation volume per se was found to be a somewhat difficult measure of quality for fMRI methods, particularly since the chosen statistical threshold ($z = 2.3$ in this case) is somewhat arbitrary. Inter-scan variability for a single subject was large, which may be at least in part attributable to a habituation of the BOLD response to the repeated gustatory

stimulation. An alternative approach for comparing the two techniques used here would be to interleave both sequences in the same activation experiment, although this would be at the expense of temporal resolution.

In the SENSE data the SNR and the SFNR were reduced by 21% and 17%, respectively, relative to the conventional spiral approach. Hence the technique should be used only when the benefit from reducing susceptibility artefacts and the gradient duty cycle outweighs SNR concerns. In the case of investigations of the chemical senses, the overall loss of stability can be more than compensated for because the regions of interest in the orbitofrontal cortex particularly benefit from reduced signal loss and blurring.

The taste stimulation activated OFC, insula cortex and the anterior cingulate, consistent with a previous study using both glucose and salt as stimuli in identical concentrations (O'Doherty et al. 2001). In contrast to this study, we did not find activation differences between the two stimuli, probably due to the very small sample size used in our study. Similar to the results obtained in the taste study described in Chapter 5, the choice of 25 mM KCl and 2.5 mM NaHCO₃ as a neutral control solution has proven somewhat problematic. Some subjects described it as having a slightly salty/bitter unpleasant taste and the BOLD responses to this stimulus were not markedly different from those obtained after administration of the taste solutions. For future studies, it might therefore prove necessary to calibrate the ionic concentration of the 'tasteless' stimulus individually for each subject.

As a final point it should be emphasized that SENSE acceleration proved especially effective in suppressing susceptibility artefact at shorter TEs than are commonly used for BOLD fMRI. Therefore, short-TE and spin echo applications, such as perfusion and diffusion imaging, are further promising candidates for spiral

SENSE acquisition. Moreover, due to enhanced susceptibility effects, imaging at higher main magnetic field strength particularly favours parallel acquisition in single-shot applications. It would also be possible to combine the passive shimming method described in the following chapter with the SENSE acquisition, resulting in optimised signal detection in OFC. Even though the SENSE technique was found superior to conventional EPI, unfortunately this technique could not be employed for the functional studies described in the following chapters, since neither parallel imaging hardware nor software was available at the FMRI scanner at the time the data was collected.

4 Passive shimming

Imaging the human orbitofrontal cortex (OFC) with fMRI is problematic due to the proximity of this region to the air-filled sinuses, which causes susceptibility artefacts. Placing a strongly diamagnetic material into the mouth ('mouthshim') of a human volunteer can significantly reduce artefacts in this region. Using the same combined olfactory and visual fMRI paradigm, the following study compared brain activation and static B_0 field maps of participants being scanned both with and without the 'mouthshim'. Results demonstrate that the device improves the B_0 field homogeneity within OFC, resulting in significantly stronger BOLD activation in this region. However, the device also caused both increased head motion and reduced activation in insula cortices due to more frequent swallowing and tactile stimulation of the tongue. The 'mouthshim' should only, therefore, be used where sensitivity in OFC regions is paramount.

4.1 INTRODUCTION

As described earlier (Chapter 1), multisensory interactions involving olfaction are likely to occur primarily within the orbitofrontal cortex (OFC), since it is a prime area of convergence for the chemical senses (Anderson et al. 2003; O'Doherty et al. 2001; Small et al. 1999b). Unfortunately, imaging this region with gradient echo (GE) methods typically employed in blood oxygenation level dependent (BOLD) functional

magnetic resonance imaging (fMRI) suffers from susceptibility artefacts. This problem is aggravated by the trend to use increasingly high field (≥ 3 Tesla) magnets for fMRI studies, since the extent of the artefacts increases with field strength (Abduljalil and Robitaille 1999; Krasnow et al. 2003).

As indicated before, various methods to reduce susceptibility artefacts have been developed, including sequence parameter optimization, gradient compensation, optimization of the slice angle and tailored RF pulses (Constable and Spencer 1999; Deichmann et al. 2003; Li et al. 2002; Ojemann et al. 1997; Posse et al. 2003). However, the use of these techniques is usually associated with one or several disadvantages such as longer experiment duration and lower spatial or temporal resolution. Recently, it has also been shown that different phase encoding schemes in conjunction with an optimised slice angle and a compensating gradient can significantly improve both signal intensity and BOLD sensitivity without negatively affecting temporal resolution (De Panfilis and Schwarzbauer 2005) supporting the finding provided by Deichmann and colleagues (2003).

Other strategies to improve signal in OFC, without altering the study parameters, seek to directly improve the local B_0 homogeneity in this region by active shimming with shimming coils (Constable and Spencer 1999; Wilson et al. 2002a).

It has recently been demonstrated that placing the highly diamagnetic Continuously Nucleated Pyrolytic Graphite (CNPG) in the roof of a subject's mouth ('mouthshim') constitutes a safe and effective passive shimming method that reduces susceptibility artefacts in OFC (Wilson et al. 2002b; Wilson and Jezzard 2003). Additionally, a protocol has been developed that allows for the optimised use of the CNPG according to the individual geometry of the subject's intrinsic B_0 distribution (Wilson et al. 2003). Using a reward-punishment task, Cusack and colleagues (2005)

have also demonstrated that the CNPG increased functional sensitivity in OFC.

In this chapter, these previous findings are extended by investigating whether the use of CNPG as a passive ‘mouthshim’ results in increased activation in OFC when employed in a functional MRI paradigm with olfactory and visual stimulation. This paradigm was specifically chosen because olfactory stimulation reliably activates OFC regions (Anderson et al. 2003; Gottfried et al. 2002a; Zald and Pardo 2000; Zatorre et al. 1992) and, conversely, visual activation in the occipital lobe should remain unaffected by improvements in frontal B_0 homogeneity. Therefore the visual stimulation was used as a control condition, because any differences in activation within the visual cortex would have to be attributed to other factors associated with the ‘mouthshim’, such as increased movement of the participants. To test for these other factors, the effect that being fitted with the ‘mouthshim’ had on subject’s head motion and the functional consequences of both increased swallowing and tactile stimulation of the tongue was also investigated.

4.2 MATERIAL AND METHODS

4.2.1 Participants

Twelve healthy right-handed volunteers participated in this study (7 females and 5 males; mean age 26 years, age range 22-31 years). Each participant was scanned twice with the same functional paradigm, but with a ‘mouthshim’ fitted during one of the scanning sessions. In order to control for session effects, half of the participants were scanned with the ‘mouthshim’ in the first session. All participants gave written informed consent after having received the instructions for the study. The first 30 functional EPI volumes of one subject (with ‘mouthshim’) had to be discarded from the study because of excessive motion during the first 1 ½ minutes of scanning so that data analysis at the group level includes one truncated dataset. The study was approved by the Central Oxford Research Ethics Committee (C99.179).

4.2.2 Material

The intra-oral passive shims (‘mouthshim’) consisted of 5.69 cm³ of Continuously Nucleated Pyrolytic Graphite (CNPG, Minteq Intl. Inc., Slippery Rock, PA), which was fully enclosed within a customized mouth mould of 1mm thick sheets of polymorph plastic (Middlesex Teaching Resources, UK). The ‘mouthshim’ was individually fitted for each participant prior to scanning.

4.2.3 Stimuli and task

During scanning, subjects were presented for 6 s with visual-olfactory stimulus pairs, followed by a rest period of 30s, during which only clean air was delivered to the subjects. A total of seven different olfactory stimuli were used for this study (apple, spearmint, orange, banana, butyric acid, galbanum oil and strawberry), all provided by Quest Intl. Digitized photos, which were judged as being congruent with the odours were used as visual stimuli. Each odour was presented twice together with a congruent picture (e.g. smell of apple with the picture of apples) and twice with an incongruent picture (e.g. smell of apple with the picture of toothpaste), resulting in a total of 28 stimulus presentations. All olfactory stimuli were delivered with a custom-built, computer-controlled olfactometer. Subjects were asked to breathe normally through their nose while an air-stream of either odorized or odourless air was delivered at a flow rate of 6 litres/s through a Teflon tube placed directly under the subject's nose. Full screen colour images were generated using a video projector located outside the scanner room and projected onto a translucent screen placed directly outside the magnet bore. Subjects wore prism glasses so that they could see the screen while lying in the scanner. The task during the scanning was firstly to detect both onset and offset of each olfactory stimulus and secondly to rate each picture-smell combination with respect to 'goodness of fit between the picture and smell' on a rating scale ranging from 1='very good' to 4='very bad'. Subjects indicated both measures by pressing one of 4 buttons. Behavioural measures for the congruence between pictures and odours were collected 15s after the end of each bimodal stimulus presentation, when subjects were visually cued to enter their rating. Analysis of the congruency ratings and BOLD response are described in Chapter 8.

4.2.4 Data acquisition and analysis

Both functional and structural MRI images were acquired using a 3T Varian Inova spectrometer (Varian Inc. Palo Alto, CA) fitted with a Magnex SGRAD head gradient coil (Magnex Scientific Ltd., Oxfordshire, UK) and a birdcage transmit/receive head coil. For each of the functional data series, a total of 340 T2* weighted echo-planar imaging (EPI) volumes were taken over a time period of 17 min. Each volume consisted of 24 continuous oblique (tilted approximately 10° upwards from the anterior to posterior commissure line, so as to be aligned with the temporal lobes) slices of 3mm thickness with an in-plane resolution of 4×4mm. The tilt angle was held constant for each subject during both experimental sessions but varied slightly between subjects. Even though dropout effects may be reduced by tilting the slice orientation towards the coronal plane (Merboldt et al. 2001), only tilt angles in the region of 30° have been demonstrated to significantly improve orbitofrontal BOLD sensitivity (Deichmann et al. 2003). In the present study, the influence of tilt angle on BOLD sensitivity should be comparatively small. These imaging parameters allowed for imaging the ventral two thirds of the brain until approximately Z-coordinate of +50 of the MNI 152 standard space, to include all primary and secondary olfactory areas in orbitofrontal cortex and temporal lobes as well as the visual cortex. Other imaging parameters were: TR=3s, 64×64 matrix, FOV 256×256 mm, TE=30ms, interecho spacing $\Delta t = 746\mu s$, flip angle = 90° and readout bandwidth = 100 kHz. Additionally, an automated global shimming method was used (Wilson et al. 2002a). After each of the two experimental sessions, a B₀ field map was acquired using a combined symmetric and asymmetric spin echo sequence.

For registration into standard space, a whole brain T2* weighted EPI volume (54 slices, TR=7s, other imaging parameters as above) and a high-resolution, whole-

brain T1 weighted morphological scan (inversion-recovery fast gradient echo, 1.5mm slice thickness, 1.5mm×1.5mm in-plane resolution) were acquired after the experimental paradigm.

Statistical image analysis of the functional dataset was carried out using FEAT, the FMRIB Expert Analysis Tool (www.fmrib.ox.ac.uk/fsl). Prior to data analysis the first 3 volumes were deleted (to avoid intensity changes due to the approach to steady state magnetization) and the following pre-processing was applied: motion correction using MCFLIRT (Jenkinson et al. 2002); spatial smoothing using a Gaussian kernel of FWHM 5mm; mean-based intensity normalization of all volumes by the same factor; non-linear high-pass temporal filtering (Gaussian-weighted LSF straight line fitting, $\sigma=36.0s$). A general linear model (GLM) using the timing of the 28 bimodal odour-picture presentations was fitted to the time course at each voxel. Statistical analysis for each experimental run was carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich et al. 2001).

For group analysis, the individual results were registered both to high-resolution anatomical MR images and to the Montreal Neurological Institute (MNI) 152 standard image. Registration to high resolution and standard images was carried out using FMRIB's Linear Image Registration Tool (FLIRT) (Jenkinson et al. 2002). The statistical analysis focused on differences between the two sessions (with vs. without 'mouthshim'). Mixed-effects (often referred to as 'random-effects') group analysis was carried out using FMRIB's Local Analysis of Mixed Effects (FLAME) software (Beckmann et al. 2003) with a cluster threshold of $Z>2.0$ and a cluster significance threshold of $P=0.05$ (corrected for multiple comparisons) (Forman et al. 1995; Friston et al. 1994; Worsley et al. 1992).

BOLD sensitivity maps (Deichmann et al. 2002) were calculated from the field gradient maps for the conditions of with and without the mouthshim present. The BOLD sensitivity index has been shown (Deichmann et al. 2002) to be given by the product of the effective echo time (TE_{eff}) and the achieved image intensity (I). These parameters depend on the gradient of the in-plane (g_y) and through-plane (g_z) field inhomogeneities, as follows:

The effective echo time, TE_{eff} , is given by $TE_{\text{eff}}=TE/Q$, where TE is the intended echo time of the center of k-space and $Q(x,y,z)$ is a dimensionless constant given by:

$$Q(x,y,z) = 1 \pm \frac{\gamma}{2\pi} \tau_{pe} FOV_y g_y(x,y,z)$$

where τ_{pe} is the echo-to-echo interval in the EPI train (746 μ s in our study), and FOV_y is the field of view in the phase encode direction. The addition or subtraction of the second term depends on whether k-space is traversed bottom-to-top or top-to-bottom, respectively.

The image intensity, I , is predominantly affected by the presence of through-plane field gradients that lead to through-plane signal dephasing, as well as by an apparent scaling of the local spin density by a factor Q , according to

$I=I_0\text{sinc}(\Delta\Phi_z(x,y,z))/Q$ (for $\Delta\Phi_z(x,y,z) < 2\pi$) and $I=0$ (for $\Delta\Phi_z(x,y,z) \geq 2\pi$). The phase spread parameter, $\Delta\Phi_z$, is in turn given by:

$$\Delta\Phi_z(x,y,z) = \gamma g_z \Delta z TE_{\text{eff}}$$

where Δz is the slice thickness (3mm in our study). Note that if TE_{eff} falls outside the acquired data window then no signal intensity is recorded from that location.

BOLD sensitivity maps were calculated from the measured maps of $g_y(x,y,z)$ and $g_z(x,y,z)$ according to the two above equations. The maps were then analyzed for differences in BOLD sensitivity in specific regions of interest.

4.3 RESULTS

4.3.1 Head motion

All participants tolerated the ‘mouthshim’ without reporting subjective feelings of discomfort. However, all subjects reported increased salivation while wearing the ‘mouthshim’. As a result of this, swallowing during the scanning sessions was likely to be more frequent, which in turn could have increased the subject’s head motion. Even though no formal measure of swallowing frequency was obtained, head motion could be quantified by the amount of motion correction applied to the experimental dataset at the pre-processing stage of image analysis. Motion due to swallowing should especially cause increased rotation around the x-axis (nodding). This is because the pivotal point for this rotation is at the back of the head, so that the whole movement corresponds to a rotation around the x-axis through the head’s centre of mass, combined with a translation in the z-direction. Because we expected increased movement in the ‘mouthshim’ condition we conducted one-tailed comparisons between the two conditions. The results of the head motion analysis demonstrated increased absolute motion (measured as the average absolute distance of the 340 functional volumes from the ‘template’ image, itself taken to be the volume acquired half way through the experimental session) for 9/12 subjects in the ‘mouthshim’ condition and this difference was significant ($\bar{O}_{\text{with ‘mouthshim’}} = 0.63 \text{ mm}$; $\sigma = 0.27$ and $\bar{O}_{\text{without ‘mouthshim’}} = 0.43 \text{ mm}$; $\sigma = 0.23$; paired t-test, $df=11$, $p \leq 0.029$). However, this measure of absolute motion quantifies the absolute displacement of every voxel in the brain, which is then averaged. Consequently, this calculation includes both translational and rotational movement and as such is a good measure for overall motion, but not specific to the swallowing movement. All twelve subjects had

significantly increased relative motion (measured as the average distance from each of the functional volumes to the next) while being scanned with the ‘mouthshim’ ($\bar{\varnothing}_{\text{with ‘mouthshim’}} = 0.094 \text{ mm}$; $\sigma = 0.029$ and $\bar{\varnothing}_{\text{without ‘mouthshim’}} = 0.076 \text{ mm}$; $\sigma = 0.019$; paired t-test, $df = 11$, $p \leq 0.029$). Detailed analysis of the amount of translational and rotational motion in each condition (Figure 4.1) confirmed that wearing the ‘mouthshim’ resulted in significantly more relative rotational movement around the x-axis ($p \leq 0.01$) and increased translational motion along the z-axis ($p \leq 0.05$).

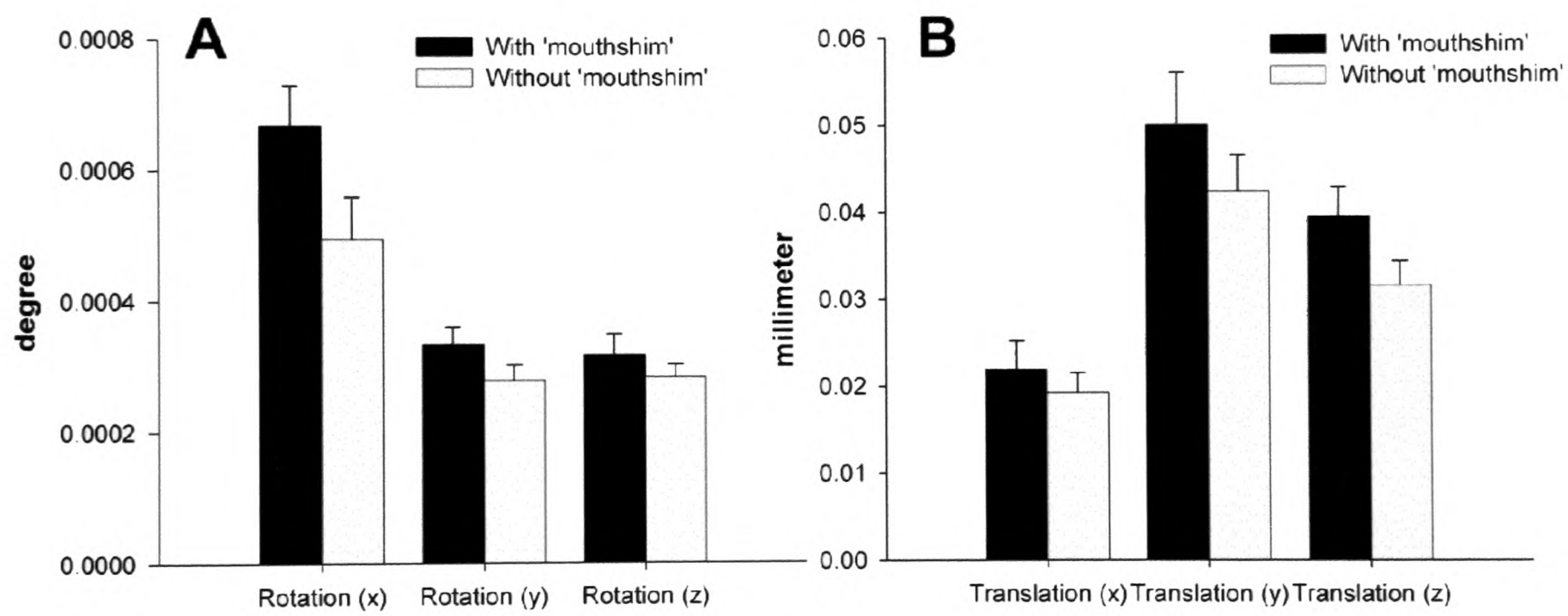


Figure 4.1: Head motion in the two experimental sessions.

Significantly ($p = 0.05$) increased rotational motion around the x-axis (A) as well as translational motion along the z-axis (B) was found when subjects were scanned with the 'mouthshim'. Data are averaged for 12 subjects and error bars indicate the standard error.

4.3.2 Olfactory-visual activation

Consistent with previous studies of the olfactory and visual system, a network of areas including the orbitofrontal cortex, insula cortices, primary visual cortex and

medial temporal regions were activated (see Table 4.1 and Figure 4.2) by the simultaneous olfactory-visual stimulation in both conditions (with and without ‘mouthshim’).

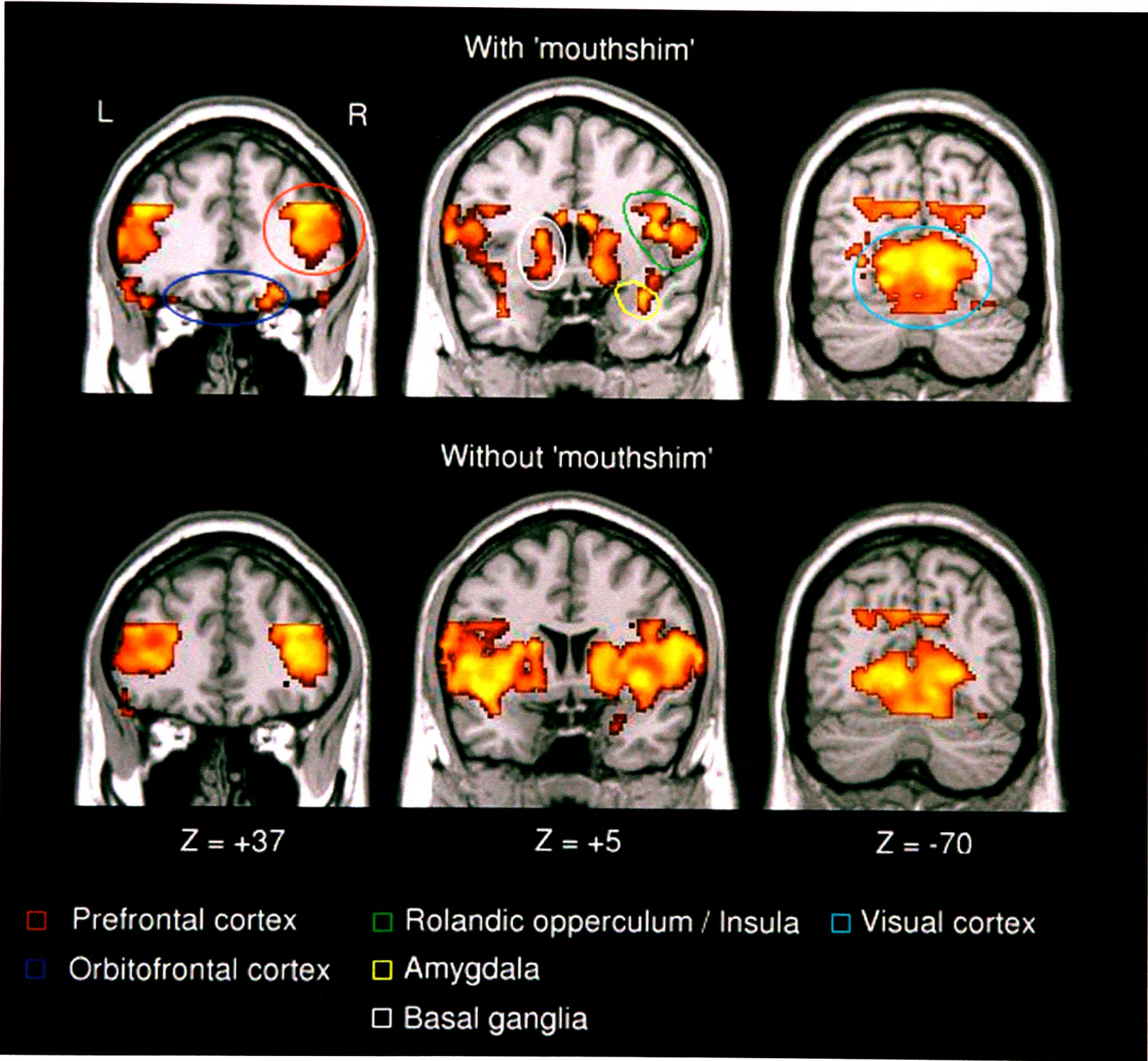


Figure 4.2: Brain activation after visual-olfactory stimulation.

The image shows the group activation ($p < 0.05$, corrected for multiple comparisons) for all 12 subjects either while wearing the passive shim (top) or without the ‘mouthshim’ (bottom). In both conditions, significant activation could be observed in the prefrontal cortex, the insula cortex/rolandic operculum, amygdala, basal ganglia and the visual cortex. Significant activation in the right OFC was observed in the ‘mouthshim’ condition only. Activated voxels are rendered into the MNI standard space and the left side of the brain is shown on the left side of the picture.

Brain region	Side	With Mouthshim		Without Mouthshim	
		MNI coordinates	Z-score	MNI coordinates	Z-score
Lateral orbital gyrus	R	40/52/-12	4.6	44/44/-20	3.6
	L	-46/46/-18	4.0	-50/40/-14	2.4
Medial orbital gyrus	R	22/42/-24	3.7	-	-
Inferior frontal gyrus	R	44/30/24	3.9	46/32/26	5.1
	L	-44/32/26	4.8	-44/36/24	3.9
Caudate	R	20/12/14	3.0	20/10/14	4.0
	L	-16/16/10	3.5	-18/12/10	3.7
Insula cortex	R	36/18/-2	3.5	46/20/-4	4.8
	L	-34/20/-2	3.4	-46/8/-8	4.8
Amygdala / piriform cortex	R	38/8/-20	3.2	26/6/-28	2.7
	L	-36/6/-22	2.8	-	-
Pallidum	R	18/2/-6	3.1	18/-2/-2	3.9
	L	-18/2/-8	2.5	-16/-4/-4	4.0
Thalamus	R	16/-14/14	3.0	18/-8/4	3.6
	L	-18/-14/12	3.1	-22/-14/8	3.3
Hippocampus	R	28/-20/-12	3.4	26/-24/-12	3.7
	L	-28/-16/-14	3.6	-34/-14/-16	3.3
Supramarginal gyrus	R	54/-42/32	3.9	-	-
	L	-52/-44/30	4.5	-50/-42/32	4.2
Lingual gyrus	R	10/-72/-4	5.1	22/-76/2	4.5
	L	-18/-72/2	4.1	-6/-76/0	5.2
Striate cortex (BA 17)	R	1/-84/2	4.1	8/-84/-4	4.2
	L	-2/-88/-2	4.5	-4/-84/0	4.7

Table 4.1: Activated Brain Regions.

The group activation after olfactory-visual stimulation is shown for both conditions separately. The coordinate and Z-score relate to the highest activated voxel in each cluster in the MNI standard space (n=12).

4.3.3 Effect of ‘mouthshim’ on brain activation

A paired t-Test comparing the effect size of the functional activation between the condition ‘with mouthshim’ and ‘without mouthshim’ revealed an area in medial orbitofrontal cortex, where activation to the olfactory-visual stimulation was significantly higher ($p < 0.05$, corrected for multiple comparisons) when subjects were fitted with the device (Figure 4.3).

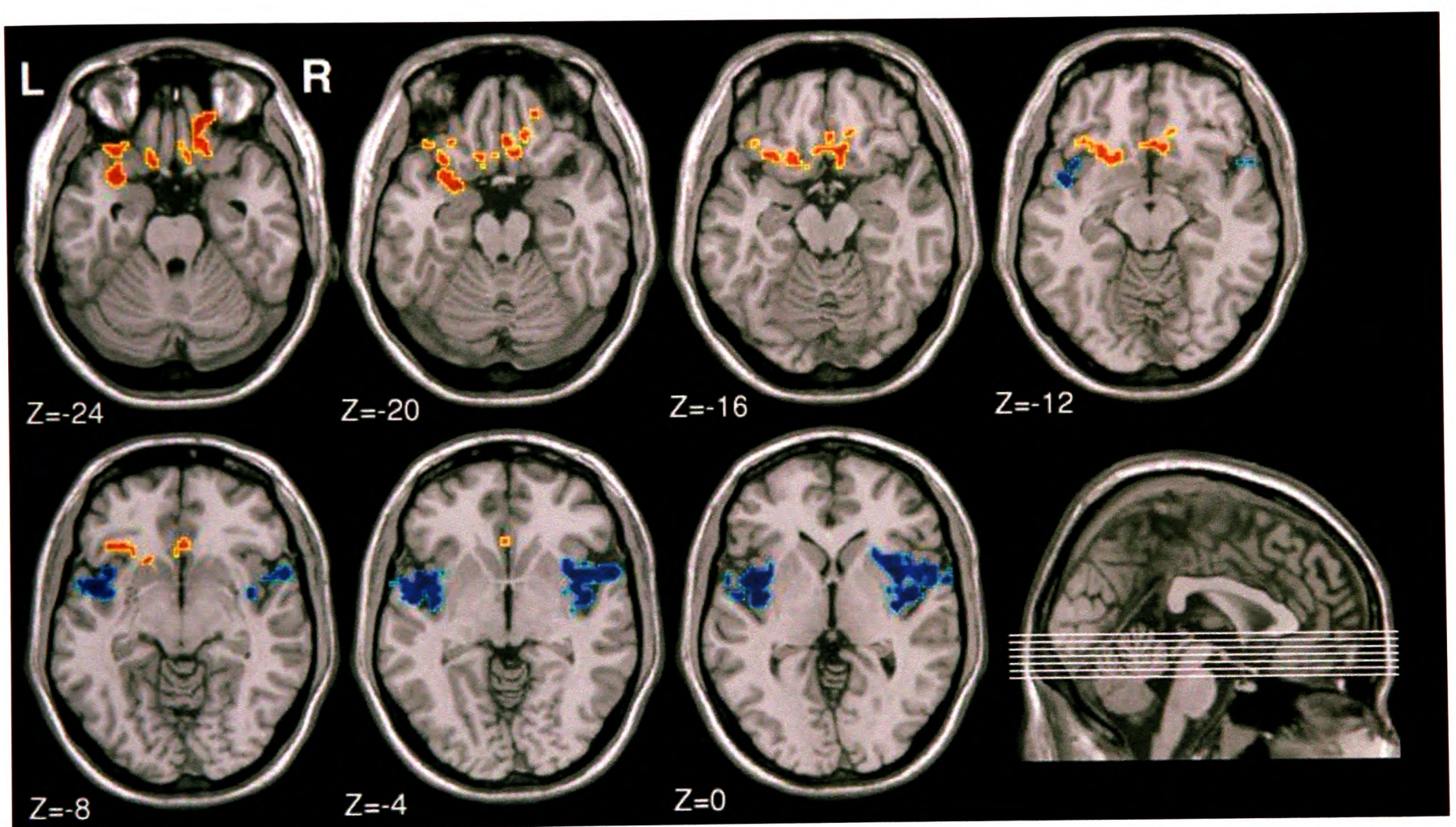


Figure 4.3: Effects of ‘mouthshim’.

Areas with significantly ($n=12$; $p < 0.05$, corrected for multiple comparisons) increased activation in the ‘mouthshim’ condition were found in both medial and lateral orbitofrontal cortex (red). In contrast, the insula cortex was more strongly activated bilaterally, when subjects had no ‘mouthshim’ (blue). Activated voxels are rendered into the MNI standard space and the left side of the brain is shown on the left side of the picture.

The peak of this difference was located in the right medial orbital gyrus ($x, y, z = 20, 46, -22$; z -score = 3.45). Additional differences were found in the right gyrus rectus ($x, y, z = 10, 26, -14$; z -score = 3.35), the left piriform cortex ($x, y, z = -24, 8, -20$; z -score = 3.29) and within the left olfactory sulcus extending into the nucleus accumbens ($x, y, z = -16, 18, -10$; z -score = 3.28). In contrast, activation in both insula cortices ($x, y, z = 42, -6, -8$; z -score = 3.81 and $x, y, z = -46, 6, -6$; z -score = 3.82) was significantly stronger when subjects were scanned without the 'mouthshim'. No differences in activation were observed within visual areas.

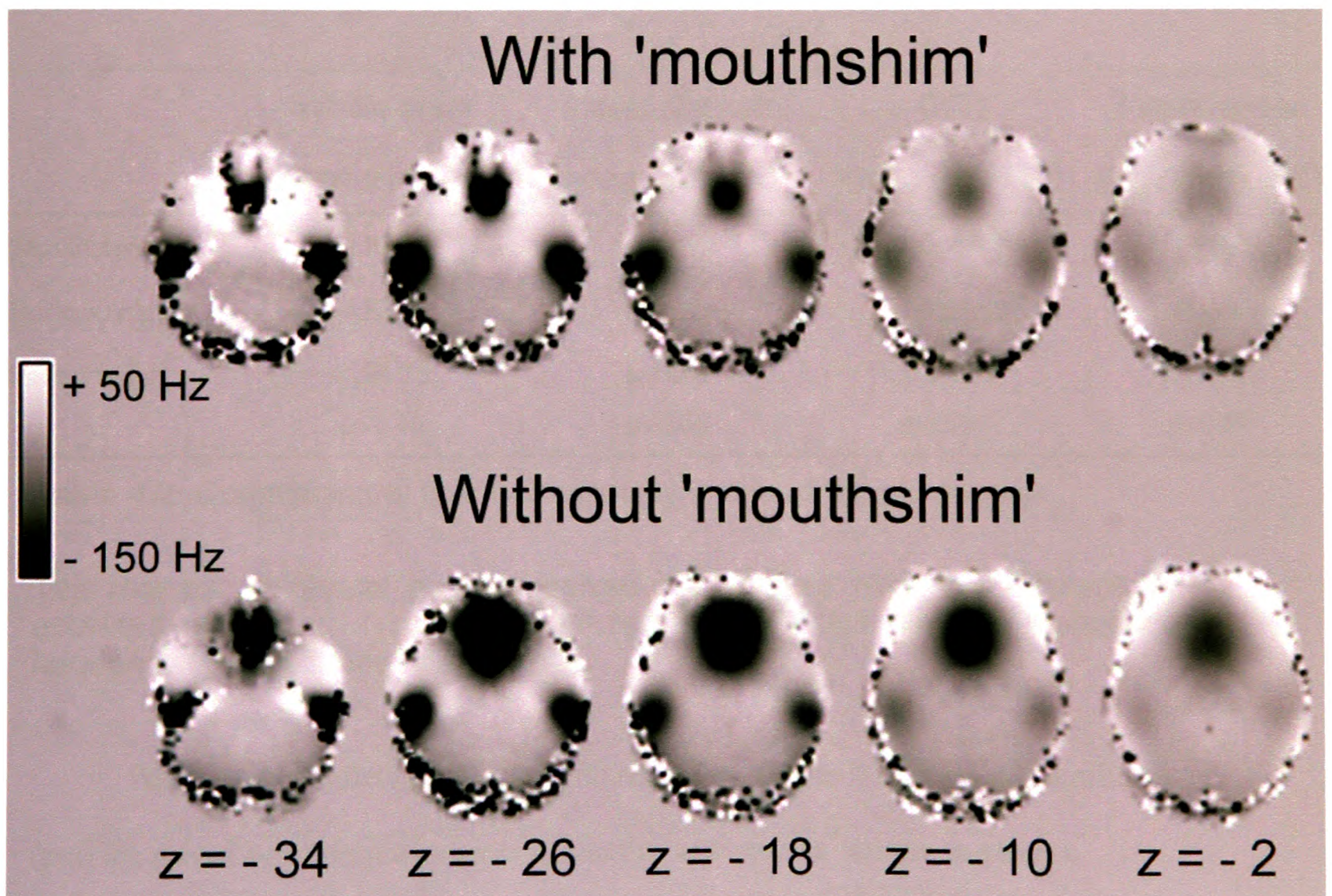


Figure 4.4: B_0 field maps.

The extent of the orbitofrontal susceptibility artifact is substantially reduced by the 'mouthshim'. The averaged B_0 field maps ($n=12$) for both conditions are shown in the MNI standard space.

In order to investigate whether these differences could have arisen from the fact that subjects had an object in their mouth in one of the experimental conditions, rather than improvements in field homogeneity, the associated B_0 field maps for both conditions were also analysed (Figure 4.4). The results confirm that the B_0 field homogeneity, measured as off-resonance frequency (Hz), was indeed significantly improved ($p<0.001$, paired t-test) only in OFC regions when subjects were fitted with the ‘mouthshim’, but not significantly different between the two conditions in the insula cortices, visual areas or when compared across the volume of the entire brain (Table 4.2).

	<i>Whole brain</i>	<i>Insula cortices</i>	<i>OFC</i>	<i>Visual cortex</i>
	mean (Hz) $\pm$ SD	mean (Hz) $\pm$ SD	mean (Hz) $\pm$ SD	mean (Hz) $\pm$ SD
‘Mouthshim’	-3.2 $\pm$ 4.2	-1.6 $\pm$ 2.8	-11.5 $\pm$ 10.1	-4.7 $\pm$ 3.8
No ‘mouthshim’	-4.5 $\pm$ 4.3	-2.4 $\pm$ 3.9	-56.6 $\pm$ 7.9	-4.6 $\pm$ 5.2
Paired t-test	$t_{11}=0.73$	$t_{11}=0.6$	$t_{11}=14.3$	$t_{11}=0.03$
	$p=0.48$	$p=0.56$	$p<0.001$	$p=0.97$

Table 4.2: Comparison of field homogeneity in different brain regions.

The regions of interest in this analysis are derived from the differentially activated areas in the two experimental conditions and off-resonance frequency (Hz) was measured (n=12).

Analysis of the field gradients in these regions (Table 4.3) revealed significant ($p<0.05$, paired t-test) improvements due to the ‘mouthshim’ for the in-plane x-gradient in the OFC only. Field gradients in the phase encoding direction (y) have a higher impact on the local BOLD sensitivity and even though the y-gradients in OFC were slightly lower in the ‘mouthshim’ condition ($\Delta B_y = 1.4$) compared to without ($\Delta B_y = 2.3$) this difference was not significant ($t_{11}=1.56$, $p=0.15$). However,

improvements in insula x- and z-gradients approached significance (p=0.1 and p=0.07 respectively).

	Whole brain			Insula cortices			OFC			Visual cortex		
	$ \Delta B_x $	$ \Delta B_y $	$ \Delta B_z $	$ \Delta B_x $	$ \Delta B_y $	$ \Delta B_z $	$ \Delta B_x $	$ \Delta B_y $	$ \Delta B_z $	$ \Delta B_x $	$ \Delta B_y $	$ \Delta B_z $
Mouthshim	6.1±1.7	6.1±1.7	8.2±2.1	1.5±1.4	1.2±1.2	2.3±1.4	1.9±1.2	1.4±1.2	3.9±2.1	1.3±0.2	2.2±0.5	2.2±0.5
No mouthshim	6.1±1.6	6.1±1.7	8.2±1.9	2.2±1.2	1.5±0.8	3.2±1.4	3.1±1.2	2.3±1.4	5.1±4.6	1.4±0.3	2.2±0.5	2.5±0.5
Paired t-test	t ₁₁ =0.07 p=0.95	t ₁₁ =0.05 p=0.96	t ₁₁ =0.05 p=0.96	t ₁₁ =1.8 p=0.10	t ₁₁ =0.97 p=0.35	t ₁₁ =1.97 p=0.07	t ₁₁ =2.15 p=0.05	t ₁₁ =1.56 p=0.15	t ₁₁ =0.72 p=0.48	t ₁₁ =1.35 p=0.20	t ₁₁ =0.13 p=0.90	t ₁₁ =1.50 p=0.16

Table 4.3: Comparison of field gradient in different brain regions.

The regions of interest in this analysis are derived from the differentially activated areas in the two experimental conditions and the average (±SD) absolute field gradients (Hz/mm) along all three axes are shown (n=12).

Additionally, the possibility that the difference in insula activation is due to slightly different ROIs in the two experimental conditions as a result of different image distortions can be discounted. The reason for this is that the mean degree of displacement across this region is very small. The values measured were −1.6 Hz and −2.4 HZ in the with and without ‘mouthshim’ condition respectively (Table 4.2) and using the formulae from Jezzard & Balaban (1995) I calculated that 21Hz off-resonance would result in a 1 pixel shift. The mean displacement in the insula cortices is hence in the region of 0.1 pixels and which would not cause the ROIs to be noticeably different between the two conditions.

To further rule out the possibility that the improved functional activation in insula cortices without the ‘mouthshim’ is due to an increase in the local TE caused

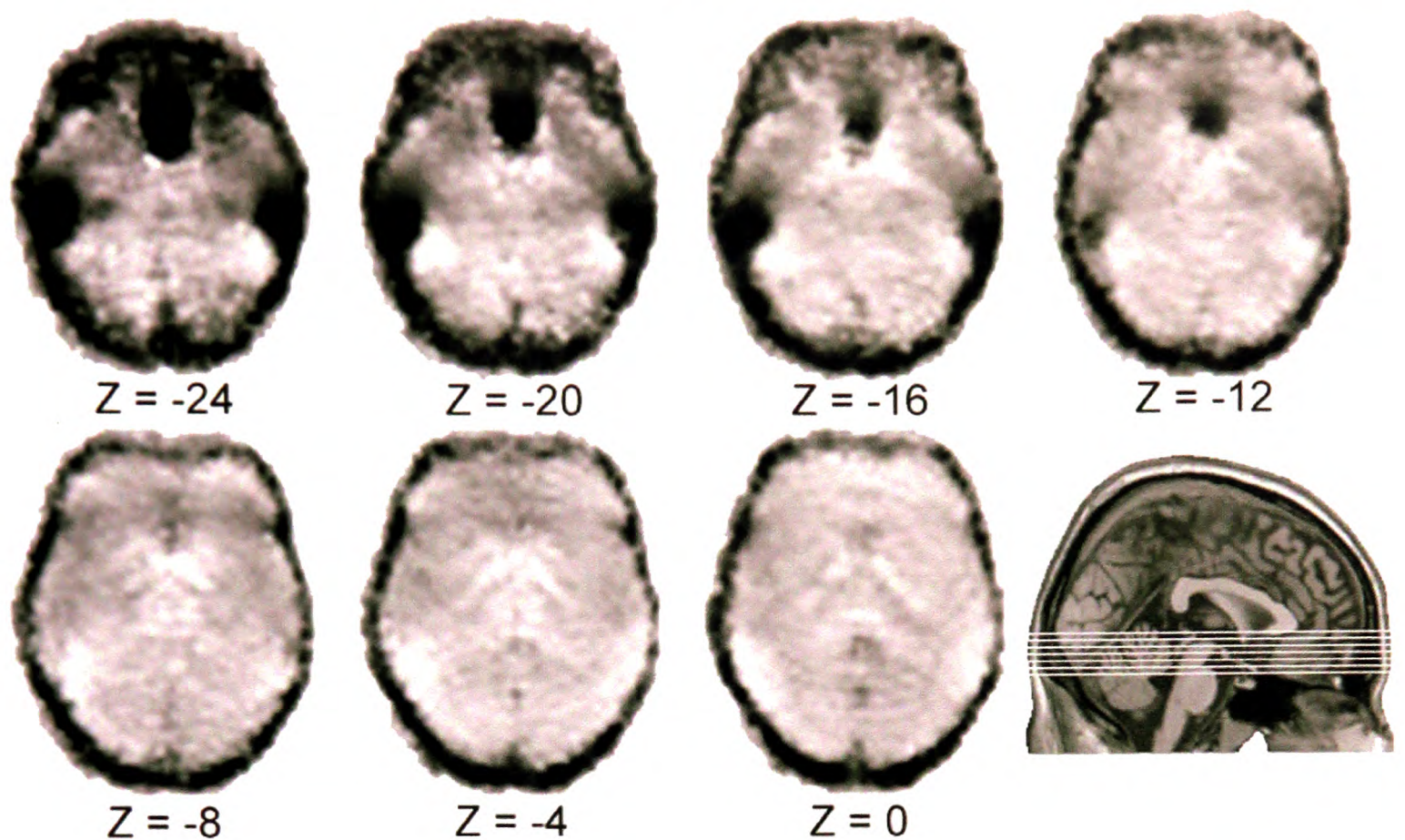
by the susceptibility gradients and consequently an increased BOLD sensitivity (BS), we analyzed the BS maps for the regions showing differences in functional activation (Table 4.4 & Figure 4.5). Significant improvements with the ‘mouthshim’ were found in OFC and when comparing the entire volume of brain scanned, but not in the insula cortices. Together, this indicates that the observed decreased functional activation in insula cortices when subjects are fitted with the ‘mouthshim’ has physiological origins.

	<i>Whole brain</i> mean %BS±SD	<i>Insula cortices</i> mean %BS±SD	<i>OFC</i> mean %BS±SD	<i>Visual cortex</i> mean %BS±SD
‘Mouthshim’	69.1±4.6	79.5±7.1	51.4±9.7	73.3±6.6
No ‘mouthshim	65.3±5.4	78.8±8.7	37.4±10.8	73.4±6.7
Paired t-test	t ₁₁ =2.97	t ₁₁ =0.27	t ₁₁ =3.54	t ₁₁ =0.05
	p=0.01	p=0.79	p=0.005	p=0.96

Table 4.4: Comparison of BOLD sensitivity (BS).

The regions of interest in this analysis are derived from the differentially activated areas in the two experimental conditions. The percentage values indicate the theoretically achievable BS, where 100% would be the theoretical BS for a sample that is perfectly shimmed throughout the relevant region (n=12).

With 'mouthshim'



Without 'mouthshim'

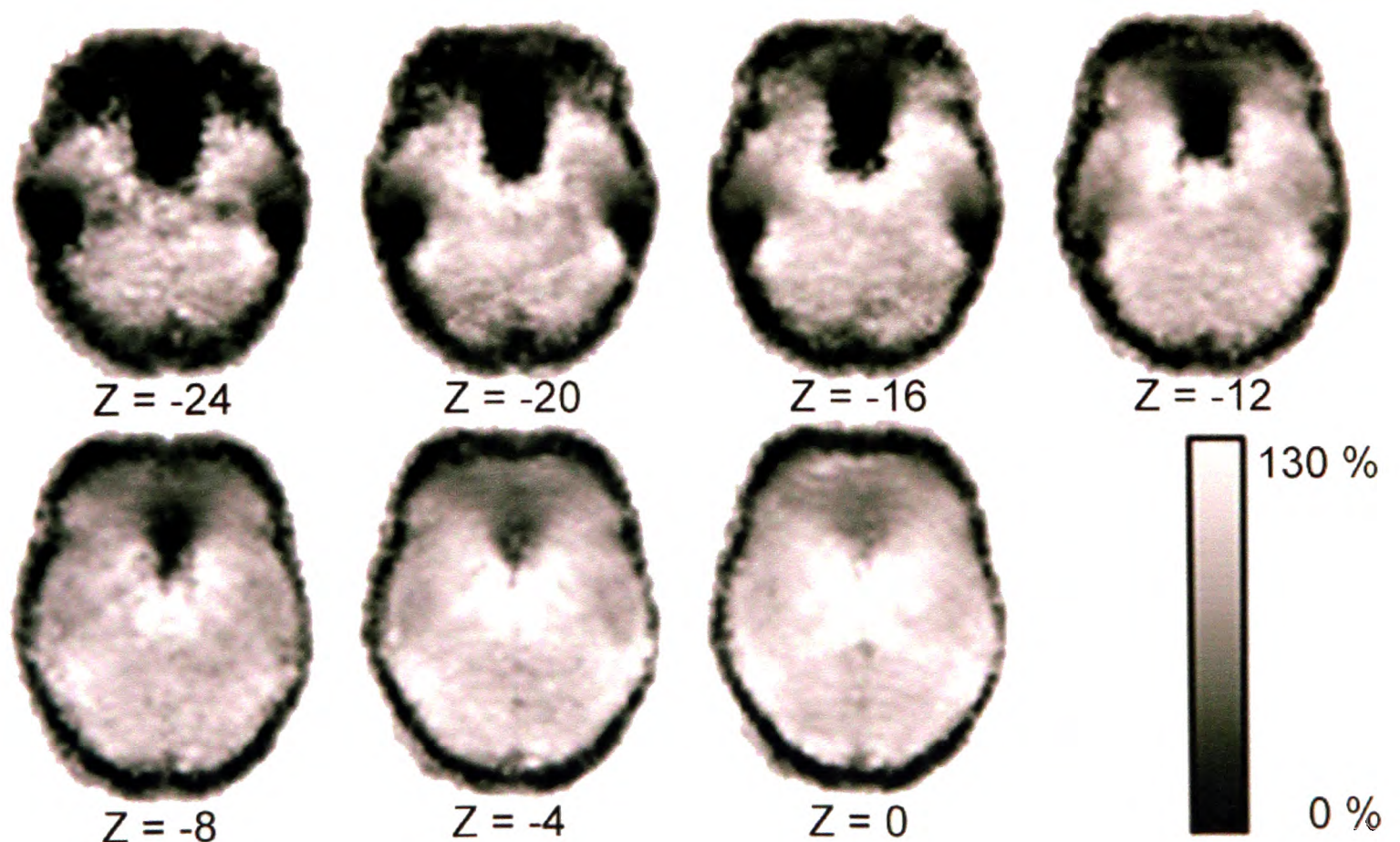


Table 4.5 BOLD sensitivity maps.

The averaged BS maps ($n=12$) for both conditions are shown for the slices that showed differences in the functional analysis and demonstrate the improvements in BS in OFC. Note that a value of 100% corresponds to a perfectly shimmed sample.

4.4 DISCUSSION

The aim of this study was to investigate the feasibility of utilising a passive ‘mouthshim’ for fMRI studies investigating OFC function by scanning participants with the same experimental paradigm while they were either fitted with the device into the roof of their mouth or without it. The device is easy to place and very tolerable to subjects, as reported by all participants. In both conditions, the visual-olfactory paradigm employed for this study resulted in activation of primary visual cortex, both insula cortices, olfactory regions in the middle temporal lobes and the OFC. Activation in these regions is consistent with other studies using visual (Tootell et al. 1998) or olfactory stimuli (Royet et al. 2001; Small et al. 2004b; Zald and Pardo 2000)

However, several differences could be observed between the two conditions. Firstly, participants moved significantly more while wearing the ‘mouthshim’. Specifically, rotational motion around the x-axis and translational motion along the z-axis was significantly increased in the ‘mouthshim’ condition. Even though no formal measure of saliva flow or swallowing frequency was employed in this study, it is highly likely that the additional motion is a result of frequent swallowing due to heightened salivation. This notion is supported by the observation that all participants reported an increase in salivation when wearing the ‘mouthshim’. Additionally, other studies have shown that the presence of a foreign object – such as dentures – in the mouth increases salivary flow up to 300% (Eliasson et al. 1996; Wolff et al. 2004). Incorporating the CNPG into a bite-bar would constitute an alternative arrangement of the device that should minimize the problem of increased head motion due to swallowing. However, the effect of increased head motion on functional activation appears to be relatively small, since no differences in activation between the two

conditions were detected within visual areas.

Secondly, significantly stronger BOLD responses in OFC were observed in the ‘mouthshim’ condition, which is likely to be a result of the improved B_0 field homogeneity in this region. The direct comparison of the associated B_0 field maps and the BOLD sensitivity between the two experimental conditions demonstrated that this improvement is specific to the OFC region, consistent with previous studies employing the device (Cusack et al. 2005; Wilson et al. 2002b; 2003; Wilson and Jezzard 2003). Additionally, these findings confirm those obtained by Cusack and colleagues (2005) by demonstrating that the ‘mouthshim’ increases functional sensitivity in OFC during BOLD imaging experiments. This suggests that the device is effective for improving image quality in fMRI studies investigating the function of the OFC.

Conversely, a significantly stronger BOLD response in both insular cortices was found when participants were not wearing the device. In a similar study using this device (Cusack et al. 2005), small reductions in anterior inferior temporal cortices as well as anterior and lateral inferior frontal cortex were observed with the ‘mouthshim’. However, the reductions observed in the present study in both insular cortices were located more posterior and superior compared to those described by Cusack et al. Additionally, the observation that off-resonance frequency, field gradients and BS in the insular cortices were either not different or slightly improved by the ‘mouthshim’ rules out field inhomogeneities as a factor responsible for decreased insula activation and strongly suggests a physiological effect. By these physiological factors I mean that the neural activity associated with movement and tactile stimulation of the tongue (not the head) will be different depending on whether there is a foreign body in the mouth or not. This in turn will heighten BOLD signals

during baseline periods in the ‘mouthshim’ condition. It is well known that swallowing as well as somatosensory stimulation of the tongue activates the insula bilaterally (Cerf-Ducastel et al. 2001; Harris et al. 2005; Mosier and Bereznaya 2001; Toogood et al. 2005). The presence of the ‘mouthim’ is therefore very likely to cause both tactile stimulation of the tongue and increased swallowing for the duration of the experiment. As a consequence, the insula will display heightened BOLD signals throughout the experiment including the baseline periods. Since the statistical analysis of the dataset consisted of a comparison between stimulation periods when odours were presented and the baseline ‘rest’ periods, the additional insula activation caused by the presence of odours may have been small compared to that elicited by the ‘mouthshim’. This in turn will mask odour related activation in this region. Therefore, I suggest that the device should be used with caution for studies investigating the combined function of the insula cortex and OFC, as would be the case for studies involving gustation (Small et al. 2004b) or pain (Rolls et al. 2003b). I also hypothesise that similar physiological effects could probably be observed when a bite-bar is used to restrain head motion. Future studies utilizing the ‘mouthshim’ should also monitor swallowing responses so that these can be explicitly included in the model for data analysis, thereby controlling for the effect the device has on insula activation.

Taken together, these results show that the placement of CNPG into the roof of the mouth significantly improves fMRI activation in inferior frontal regions of the brain, which demonstrates the feasibility of this device particularly for studies concerning OFC function. However, researchers planning to use the device should also take into account that it causes activation of both insula cortices as a result of both increased swallowing and tonic tactile stimulation of the tongue.

5 Retronasal Olfactory Stimulation

The integration of taste and smell which gives rise to the flavour percept is one of the strongest multisensory phenomena in humans and has been the subject of investigation in several fMRI studies. However, in previous studies using flavour stimuli, subjects were required to hold the stimuli in their mouths during the ‘activation’ phase of the experiment with the subsequent swallowing of the stimuli being discarded from the image analysis. While such an experimental design has several advantages (i.e. absence of stimulus-correlated motion), it also lessens the contribution of retronasal olfactory stimulation that only occurs when either strong masticating movements or swallowing take place. In the present chapter I compared two experimental fMRI designs in which subjects either swallowed a liquid flavour stimulus (sweet banana) immediately after stimulus presentation or held it in their mouths for 9s.

5.1 INTRODUCTION

Although the perception of odours can be modulated by visual cues (see Chapter 6,7&8), the most robust multisensory effects involving olfaction are observable during flavour perception, when both olfactory and gustatory inputs merge to form a flavour percept. Indeed, the interaction between olfaction and gustation is so compelling that people frequently comment on the ‘taste’ of food and drinks, even

though approximately 80% of the perceived flavour originates from the olfactory components rather than the gustatory ones (Murphy et al. 1977; Rozin 1982).

Two predominant multisensory mechanisms have been described to account for the interaction between the chemical senses: superadditivity and spatial co-localisation. The phenomenon of superadditivity has been observed both behaviourally (Dalton et al. 2000) and in fMRI studies (de Araujo et al. 2003c; Small et al. 2004b). For example, Dalton and colleagues (2000) demonstrated that the olfactory detection threshold for benzaldehyde (almond) was lowered when subjects had a congruent (sweet tasting saccharin) taste solution in their mouth, but remained unaffected when incongruent (water or MSG) tastes were used. Therefore, the adding a congruent taste to a flavour stimulus increases the perceived intensity of the olfactory component. The brain regions processing the converging inputs from the gustatory and olfactory system appear to be amygdala, frontal operculum, insula cortex and OFC, since they respond to stimulation in both modalities (de Araujo et al. 2003c; Small et al. 2004b). Additionally, superadditive effects for congruent flavours have been observed in the anterior cingulate cortex, insula cortex, frontal operculum and the caudal OFC (Small et al. 2004b). However, in the study by de Araujo and colleagues (2003), only the left anterior OFC displayed superadditive effects, where significantly stronger activation could be observed in response to the congruent combination (sweet and strawberry odour) compared to the sum of the unimodal activations to the two stimuli. It should also be noted that in the first PET study on flavour perception, Small and colleagues (1997) did not report superadditive effects, but rather showed neural suppression in primary gustatory and secondary gustatory and olfactory cortices for flavour stimuli compared with the unimodal presentations of their olfactory and gustatory components. Whether these observations are a result of

the different imaging technique used (PET) or of the stimuli employed in this study, remains to be determined. However, the authors of this study speculate that this blood flow decrease may represent the neuronal correlate of a behavioural effect observed in human psychophysical experiments, where the sum of intensity estimates to a taste and smell presented in isolation is consistently higher than the intensity of the same taste and smell presented together as a flavour. An additional factor explaining the absence of superadditive effects in this study may be the concentration of stimuli well above threshold. It is well known that according to the principle of inverse effectiveness (Stein and Meredith 1993), superadditive effects are mostly observed when stimuli are presented near threshold level. Furthermore, the manner in which stimuli were presented in this study may have been suboptimal: during bimodal trials, participants had a piece of filter paper soaked with pure tastant in their mouths whilst an odour-saturated Q-tip was waved under their noses, resulting in orthonasal olfactory stimulation. Particularly since subjects had their eyes open during stimulus presentation (and could therefore see the Q-tip being waved), this set-up may have inhibited effects of multisensory integration between the chemical senses, because odour and taste stimuli may have been perceived as emanating from different sources under these conditions and it is well known that discordant multisensory stimuli cause response depression (e.g. Stein, 1998).

The second multisensory phenomenon involving the chemical senses concerns the perceived spatial origin of an odour. Depending on whether an odour reaches the olfactory epithelium via the nostrils (orthonasally) or via the mouth (retronasally) the odour is perceived to emanate from either the surrounding air or the mouth (Rozin 1982). In the case of retronasal olfaction, this phenomenon gives rise to the so called 'taste-smell confusion', whereby subjectively the odour of a food or drink appears to

emanate from the mouth rather than the nose. The first neuroimaging study to investigate this phenomenon has recently been published (Small et al. 2005). In their study, the authors presented the same odours either ortho- or retronasally via tubes inserted into the nose, with one tube ending at the external nares and the other tube ending at the nasopharynx. A region at the base of the central sulcus - corresponding to the region responsive to oral cavity somatosensory stimulation in humans, was consistently more activated by retronasally delivered odours for all odours used. Additionally, for the food-related odour (chocolate) only, stronger retronasal activation could also be observed in medial OFC, perigenual cingulate, superior temporal gyrus and posterior cingulate cortex. Conversely delivering the same odour orthonasally resulted in stronger activation in thalamus, right lateral orbital gyrus, right hippocampus, frontal operculum and both insula cortices. Taken together these results demonstrate that neural responses elicited by an odorant are influenced by its route of administration.

Despite the clear suitability of the chemical senses for investigating phenomena of multisensory integration, comparatively few neuroimaging studies have been conducted, presumably because of the difficulties associated with stimulus delivery. In current fMRI experiments on taste or flavour using liquid stimuli, the liquid is delivered to the subject either in one bolus (de Araujo et al. 2003a; de Araujo et al. 2003c; O'Doherty et al. 2001) or over a time period of a few seconds (Cerf-Ducastel and Murphy 2001; Small et al. 2003). Following stimulus delivery, subjects are asked to 'taste' the liquid for a period of approximately 10 seconds by moving it around their mouth with their tongue. During this time, the blood oxygenation level dependent (BOLD) signal is collected, which peaks approximately 6 seconds after the onset of the stimulation. After this time, subjects are cued (by either visual or auditory

cues) to swallow the liquid and data collected during swallowing are not included in the activation analysis. The primary reason for this experimental design (i.e. to dissociate the swallowing phase from the tasting phase) is that swallowing might introduce two potential confounds to brain activation as measured by fMRI:

First, stimulus correlated head motion can lead to changes in signal intensities particularly in regions of tissue air boundaries such as the OFC, as discussed in previous chapters. This in turn can cause 'bogus' activation, i.e. BOLD signal changes that are only due to the subject's head motion and not caused by the stimulus properties. If head motion is dissociated from the time the stimulus is delivered by allowing subjects to swallow only after images from the tasting have been collected, then head motion can be modelled as a variable of no interest and does not 'contaminate' the activation. This method of stimulus presentation is clearly desirable if only pure tastants are used in the absence of any olfactory components. Despite the obvious advantages of dissociating the taste response from the swallowing response, there is a major disadvantage in this method of stimulus delivery, namely the fact that retronasal olfaction depends to a large extent on the swallowing movement which pushes the volatiles present in the mouth onto the olfactory epithelium via the nasopharynx (Buettner et al. 2001). Obviously, the natural consumption of food and drinks involves such swallowing movements and stimulus perception may to a large extent depend on these. Additionally, it could be argued that the localisation of an odour as coming from the mouth depends to some extent on the synchronicity of swallowing and olfactory stimulation in the same way that 'sniffing' causes the odour to be localised to the surrounding environment. Passively holding a flavour stimulus in the mouth will therefore result in a reduction of the olfactory components of the stimulus because the main volatile release and associated increase in perceived

flavour intensity does only occur at the time the stimulus is swallowed or when vigorous mouth movements performed (Buettner et al. 2001; Burdach and Doty 1987).

The second reason for not allowing subjects to swallow the stimulus until after the relevant data has been collected is that motor system activity due to swallowing may potentially mask activation in response to the flavour. It is well known that any voluntary movement such as swallowing activates a variety of brain areas, most prominently the motor cortex, supplementary motor area (SMA), cerebellum, basal ganglia and the insula cortex (Dziewas et al. 2003; Martin et al. 2004; Suzuki et al. 2003), which could make the dissociation of taste activation from motor activation problematic. Additionally, BOLD signal changes in response to stimulation of the chemical senses are typically extremely small at approximately 0.5% or lower (Castriota-Scanderbeg et al. 2005; de Araujo et al. 2003c; Ogawa et al. 2005; Small et al. 2003) and may be masked by those observed after swallowing and tongue movements that also appear to engage a similar neural circuitry (Martin et al. 2004; Suzuki et al. 2003). One way around this problem is to include a control condition, where subjects are ingesting a 'tasteless' control liquid. The subtraction of this control condition from the taste condition should then eliminate all somato-sensory and motor activation associated with the task. The choice of control stimulus is not uniformly agreed upon, with some neuroimaging experiments using water (Cerf-Ducastel and Murphy 2001; Ogawa et al. 2005; Schoenfeld et al. 2004), while others employ a liquid consisting of water with KCl and NaHCO₃ in ionic concentrations similar to those found in saliva (de Araujo et al. 2003a; Kringelbach et al. 2003; O'Doherty et al. 2001; Small et al. 2003). The choice of water as a control stimulus appears somewhat problematic because it was recently discovered that water itself activates regions in

the human taste cortex that are similar to those activated by pure tastants such as salt and glucose (de Araujo et al. 2003b).

The aim of this study was to investigate the feasibility of rapid delivery of liquid flavours, immediately followed by swallowing as a method for fMRI investigations of flavour perception and compare this to the methods currently used by other researchers (i.e. where swallowing occurs after the taste responses have been collected). I presented a liquid flavour stimulus (a mixture of amyl acetate and sucrose) and a tasteless control liquid to the same population of participants in two fMRI experiments using these two different activation paradigms. The data analysis was particularly concerned with investigating potential differences in olfactory regions in the two paradigms, since the retronasal component should be higher following the swallowing of the stimuli. Of additional interest was the question to what extent both motor activation as well as the head movements produced by swallowing would reduce the observable neural activation.

5.2 METHODS

5.2.1 Participants

Twenty one healthy right-handed volunteers participated in this study (8 females and 13 males; mean age 27 years, age range 20-45 years). Each participant was scanned twice with the two functional paradigms. During one of the scanning sessions subjects had to swallow the stimuli immediately after delivery ('swallow' condition), during the other session they had to hold the stimuli in their mouths for 9s ('hold' condition) before swallowing. In order to control for session effects, half of the participants were scanned using the 'swallow' paradigm in the first session. All participants gave written informed consent after having received the instructions for the study and were paid for their participation. One subject had to be discarded from the study because of excessive motion during both scanning sessions, thus data analysis at the group level includes a total of 40 datasets (20 subjects). The study was approved by the Central Oxford Research Ethics Committee (C99.179).

5.2.2 Stimuli and task

Two liquid stimuli were used for the experiments. These consisted of a flavour solution made with 1M glucose and 0.01% amyl acetate (banana flavour) in deionised water and a control solution consisting of 25mM KCl and 2.5mM NaHCO₃ in deionised water. Both liquid stimuli were delivered over a period of 3s in quantities of 3 ml into the subject's mouth via separate polyethylene catheter tubes (Vygon, Ecouen, France) with the following dimensions: $\varnothing_{\text{outside}}=2\text{mm}$, $\varnothing_{\text{inside}}=1\text{mm}$ and length=2m. Both tubes were attached to a 3-way valve system that included a syringe

and a reservoir containing the liquid stimuli. The syringes were manually operated by the experimenter who drew the required amounts of liquid from the reservoirs and delivered it to the subject. The experimenter was visually cued via a computer-controlled screen (not visible to the subject) to the onset of each stimulus presentation.

Subjects were visually cued as to when they were required to swallow by a fixation cross that changed colour from green to red for 3s and which was presented on a projector screen placed outside the scanner bore. For the 'hold' experiment, subjects were required to hold the stimulus in their mouths for 9s before they were visually cued to swallow it (Figure 5.1). This was followed by a 3s rest period before the control stimulus was delivered. For the 'swallow' experiment, the swallowing cue was presented immediately after stimulus delivery and was followed by a rest period of 12s. In both experiments, the delivery of the flavour solution alternated with that of the control solution in blocks lasting 18 seconds each and each stimulus type was presented 20 times.

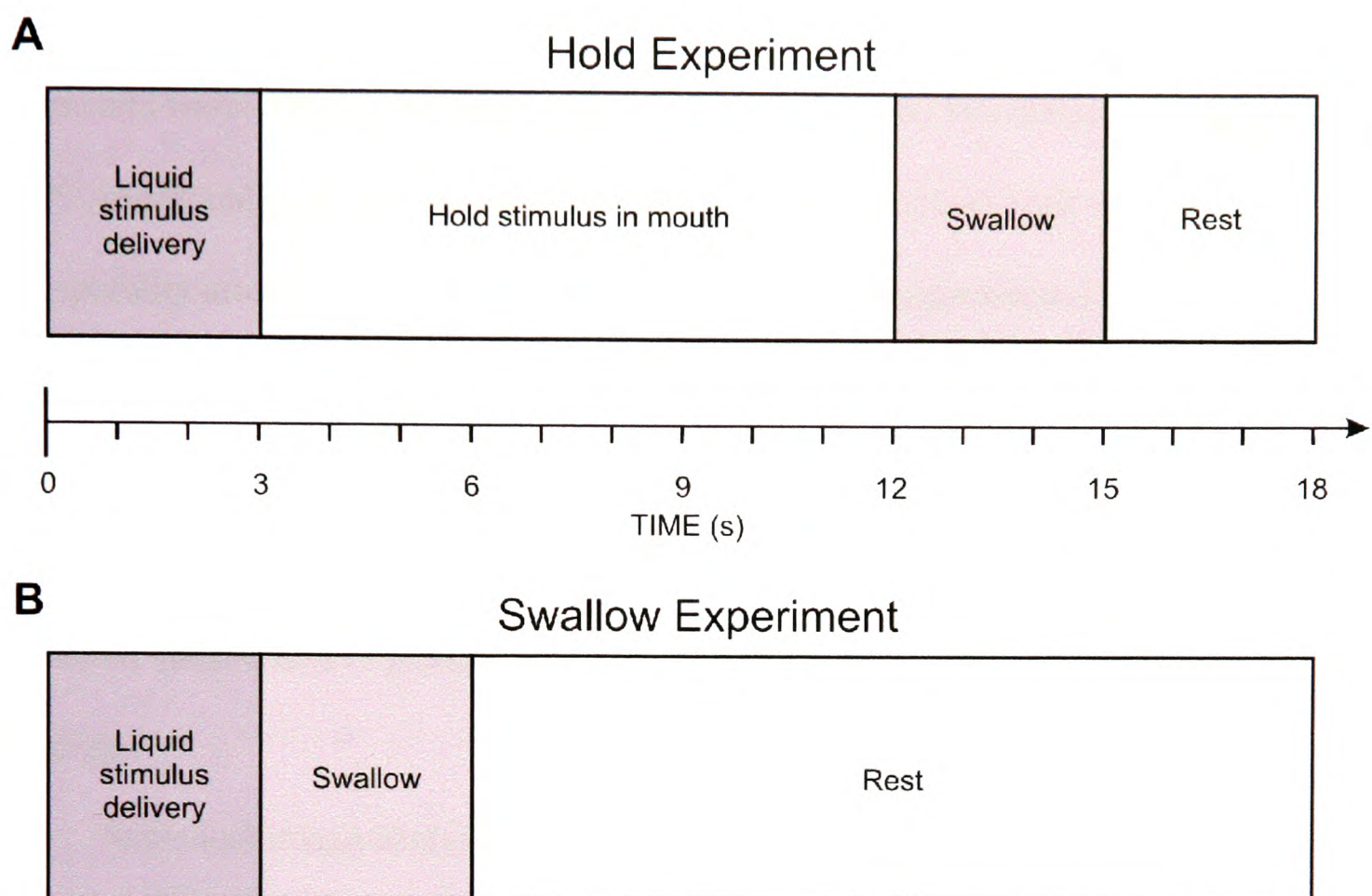


Figure 5.1: Experimental paradigm.

A The ‘hold’ conditions where subjects were required to hold the stimulus in their mouth for 9s after stimulus delivery before being cued to swallow.

B The ‘swallow’ condition, where subjects were required to swallow the stimuli immediately after their delivery.

In both experimental paradigms each stimulus block had a length of 18s and the flavour and control trials were presented in alternating order.

5.2.3 Data acquisition and analysis

Both functional and structural MRI images were acquired using a 1.5T Sonata Siemens system fitted with a head coil (Siemens Medical Solutions, Erlangen, Germany) based at the University of Oxford Centre for Clinical Magnetic Resonance Research (OCMR). For each of the two functional data series, a total of 240 T2* weighted echo-planar imaging (EPI) volumes were taken over a time period of 12

min. Each volume consisted of 35 continuous axial slices of 3mm thickness with an in-plane resolution of 3×3mm, covering the entire cerebrum. Other imaging parameters were: TR=3s, 64x64 matrix, FOV 192×192 mm, TE=30ms and, flip angle = 90°. Additionally, an automated frontal shimming method was used to minimise susceptibility artefacts in the OFC. After each of the two experimental sessions, a B₀ field map was acquired using a combined symmetric and asymmetric spin echo sequence. For registration into standard space, a high-resolution, whole-brain T1 weighted morphological scan (inversion-recovery fast gradient echo, 1 mm slice thickness, 1mm×1mm in-plane resolution) was acquired after the experimental paradigm.

Statistical image analysis of the functional dataset was carried out using FEAT, the FMRIB Expert Analysis Tool (www.fmrib.ox.ac.uk/fsl). The following pre-processing was applied: motion correction using MCFLIRT (Jenkinson et al. 2002), with the amount of motion correction applied in each session being used as a general indicator of subject's head motion; spatial smoothing using a Gaussian kernel of FWHM 5mm; mean-based intensity normalization of all volumes by the same factor; non-linear high-pass temporal filtering (Gaussian-weighted LSF straight line fitting, sigma=25s. A general linear model (GLM) using the timing of the 20 flavour presentations and the 20 control presentations as separate explanatory variables was fitted to the time course at each voxel. Statistical analysis for each experimental run was carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich et al. 2001).

For group analysis, the individual results were registered both to high-resolution anatomical MR images and to the Montreal Neurological Institute (MNI)

152 standard image. Registration to high resolution and standard images was carried out using FMRIB's Linear Image Registration Tool (FLIRT) (Jenkinson et al. 2002).

Mixed-effects (often referred to as 'random-effects') group analysis was carried out using FMRIB's Local Analysis of Mixed Effects (FLAME) software (Beckmann et al. 2003) with a cluster threshold of $Z > 2.3$ and a cluster significance threshold of $P = 0.05$ (corrected for multiple comparisons) (Forman et al. 1995; Friston et al. 1994; Worsley et al. 1992). The statistical analysis at the group level focused on the regions and strength of activation in response to both the flavour and control solutions with an emphasis on the contrast [FLAVOUR – CONTROL]. Additionally, a paired t-test was used to compare the two experimental sessions 'swallow' and 'hold'.

5.3 RESULTS

5.3.1 Head motion

Head motion was estimated from the amount of motion correction applied to each dataset at the pre-processing stage of data analysis. The average absolute motion (defined as the absolute distance of each functional volume to a reference volume taken halfway through the scan) was not significantly different between the two experimental conditions ($\bar{\Delta}_{\text{Swallow}} = 0.67\text{mm}$, $\sigma = 0.45$ and $\bar{\Delta}_{\text{Hold}} = 0.71\text{mm}$, $\sigma = 0.36$; paired t -test, $df=20$, $p = 0.71$; see Figure 5.2).

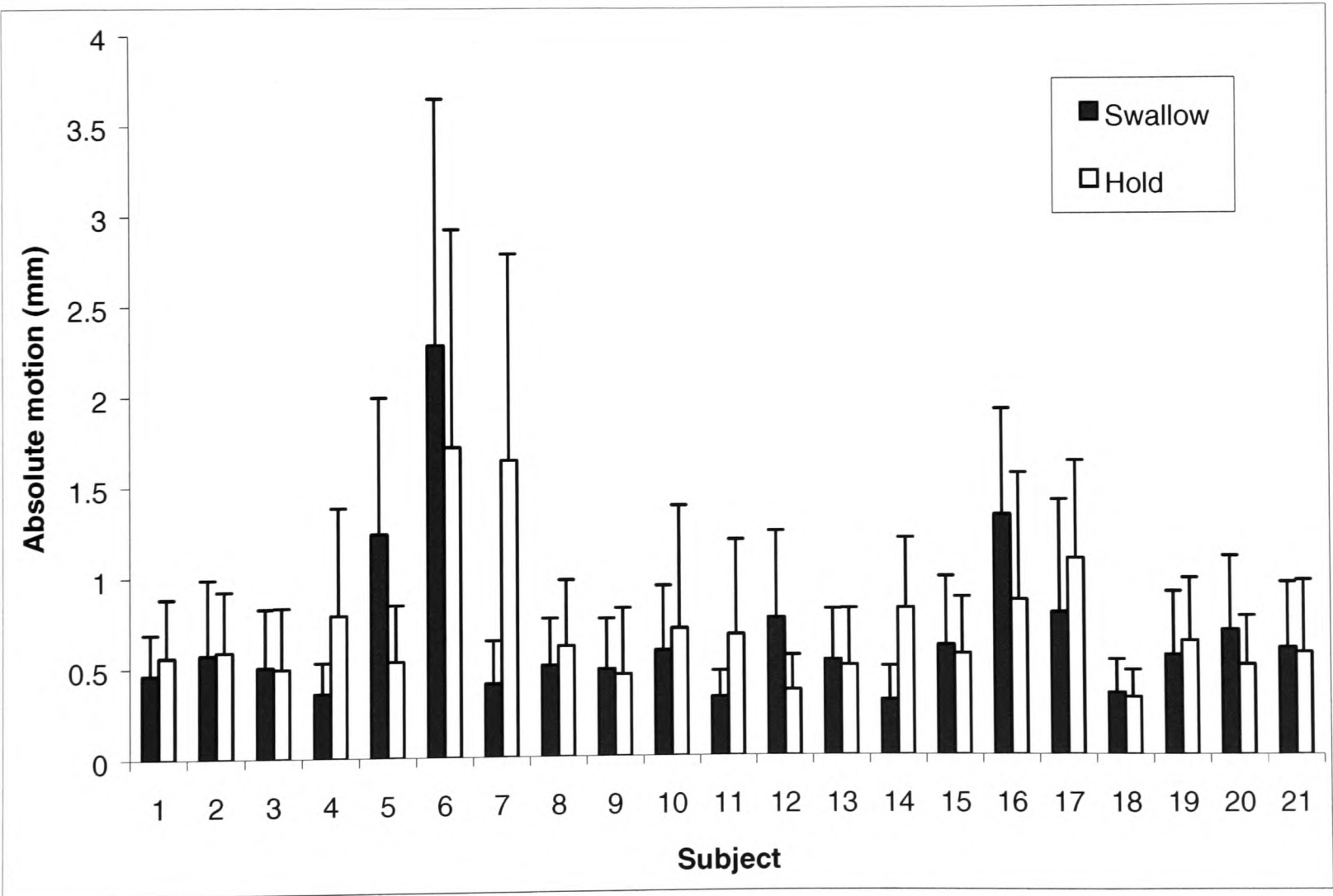


Figure 5.2: Absolute head motion.

The figure shows the absolute amount of head motion during the two experimental conditions for each of the 21 subjects. Error bars indicate the standard deviation.

Similarly, the amount of relative motion (defined as the shift from each functional volume to the next) did not differ significantly between the two experimental conditions ($\varnothing_{\text{Swallow}} = 0.102\text{mm}$, $\sigma = 0.07$ and $\varnothing_{\text{Hold}} = 0.107\text{mm}$, $\sigma = 0.06$; paired t -test, $df=20$, $p = 0.66$).

However, there were significant differences in the time intervals when the motion occurred during the stimulation blocks (Figure 5.3). A comparison of the two halves of each stimulation block (i.e., the first 9s of each stimulus presentation containing the ‘activation’ period versus the second 9s of each stimulus presentation containing the ‘rest’ period) revealed that relative head motion was significantly higher in the first half during the ‘swallow’ experiment ($\varnothing_{\text{first half}} = 0.14\text{mm}$, $\sigma = 0.11$ and $\varnothing_{\text{second half}} = 0.06\text{mm}$, $\sigma = 0.04$; paired t -test, $df=20$, $p < 0.001$) and during the second half of the ‘hold’ experiment ($\varnothing_{\text{first half}} = 0.06\text{mm}$, $\sigma = 0.03$ and $\varnothing_{\text{second half}} = 0.15\text{mm}$, $\sigma = 0.09$; paired t -test, $df=20$, $p < 0.001$). Not surprisingly, these periods of increased head motion coincided with the time when the subjects were visually cued to swallow the liquid.

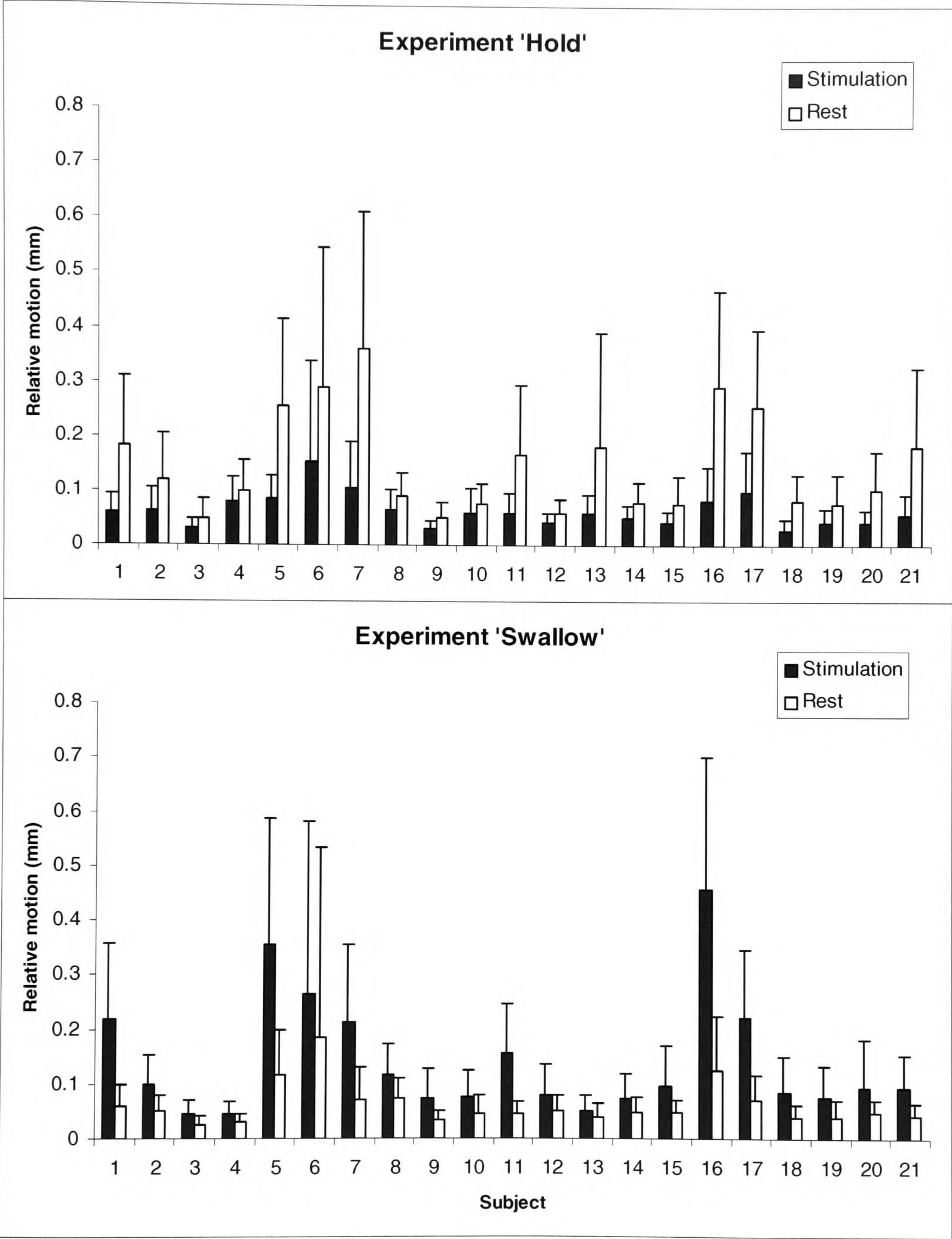


Figure 5.3: Motion comparison between stimulation and rest periods.

The amount of relative motion was significantly higher during the stimulation periods in the ‘swallow’ experiment. Conversely, subject’s head movements were higher during the rest periods in the ‘hold’ experiment.

5.3.2 Hold experiment

Both flavour and control liquids resulted in overlapping patterns of activation when compared to the rest periods, particularly within the frontal and orbitofrontal cortices (Figure 5.4). The only region found to be exclusively activated during the flavour trials was the right piriform cortex / amygdaloid region. Conversely, the control stimulus alone elicited bilateral activation in the medial temporal gyri. Nearly all of the observed activation occurred bilaterally, with the exception of the left precentral gyrus and right piriform cortex / amygdaloid region that only activated in the right hemisphere (Table 5.1).

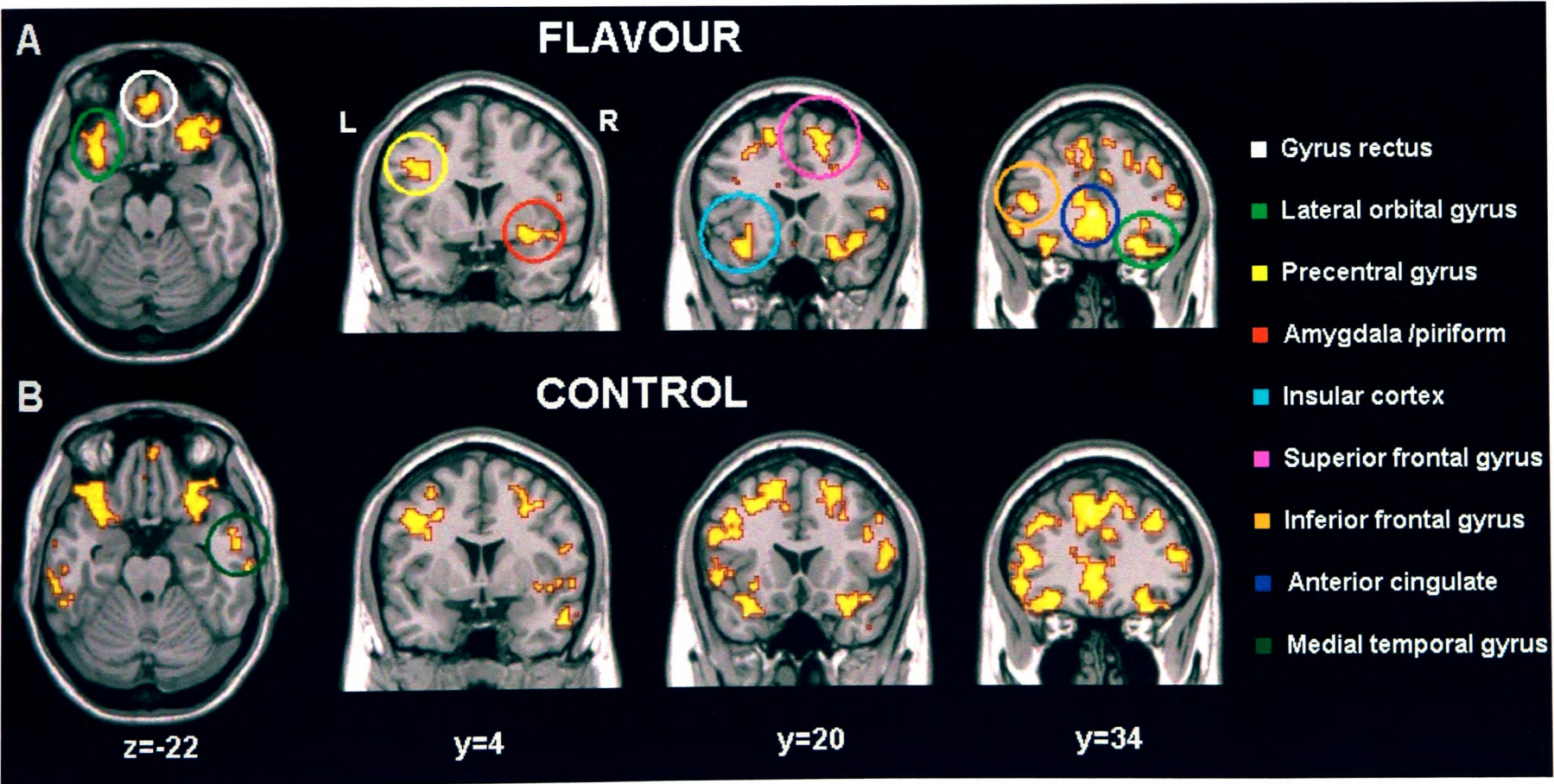


Figure 5.4: Flavour and control activation maps for ‘hold’ experiment.

Group activation maps (n=20) for both the contrasts [FLAVOUR – REST] (A) and [CONTROL – REST] (B) stimulus are shown in the canonical MNI 152 standard space. Both types of stimuli resulted in highly similar activation patterns with the exception of the piriform cortex and the medial temporal gyri that are only activated in the flavour or control conditions respectively. The left side of each slice corresponds to the left side of the brain.

Brain region	Side	<i>Flavour</i>		<i>Control</i>	
		MNI coordinates	Z-score	MNI coordinates	Z-score
Gyrus rectus	R	6/50/-14	4.26	1/50/-12	4.61
	L	-6/50/-8	4.63	-1/48/-12	4.83
Anterior cingulate	R	2/34/4	4.72	2/48/14	4.33
	L	0/34/4	5.04	0/48/16	4.53
Lateral orbital gyrus	R	28/34/-16	4.62	28/36/-16	4.29
	L	-30/36/-16	4.34	-28/34/-16	4.31
Inferior frontal gyrus	R	54/36/10	4.48	50/34/12	3.90
	L	-46/48/8	4.50	-46/46/6	4.96
Medial frontal gyrus	R	40/32/26	3.69	38/32/32	4.20
	L	-20/48/26	4.14	-42/32/30	4.02
Superior frontal gyrus	R	20/22/50	3.89	20/12/54	4.14
	L	-16/20/56	3.81	-14/20/56	3.88
Anterior insula	R	38/18/-12	4.16	32/20/-18	4.16
	L	-32/18/-20	4.26	-34/20/-16	4.31
Precentral gyrus	L	-38/10/35	4.34	-44/4/36	4.22
Piriform cortex / Amygdala	R	31/5/-10	3.36	-	-
Putamen	R	26/2/-4	3.89	26/2/0	3.14
	L	-30/4/-6	3.02	-28/-2/-6	3.24
Rolandic opperculum	R	62/-22/22	5.15	62/-16/16	4.12
	L	-58/-20/18	4.39	-56-22/16	4.16
Supramarginal gyrus	R	62/-30/36	4.86	66/-18/24	4.49
	L	-54/-40/38	3.87	-62/-22/34	4.14
Medial temporal gyrus	R	-	-	62/-30/-12	4.39
	L	-	-	-52/-12/-14	3.91

Table 5.1: Localisation of activation for ‘hold’ experiment.

The group activation shown separately for both flavour and control condition. The coordinate and Z-score relate to the highest activated voxel in each of the clusters in the MNI standard space (n=20).

In order to reveal brain regions involved in the processing of the gustatory and olfactory components of the flavour stimulus but not in the somato-sensory processing of the liquid stimuli or tongue movements, the contrast [FLAVOUR – CONTROL] was computed. Activation was restricted to comparatively small areas predominantly in the right hemisphere (Figure 5.5). Lateralised activation was observed in the right

medial ($x, y, z = 24/36/-20$; Z-score = 2.65) and lateral ($x, y, z = 30/44/-2$; Z-score = 2.88) OFC. Additionally, activation was detected the right piriform cortex ($x, y, z = 26/4/-12$; Z-score = 2.69) and adjoining amygdala ($x, y, z = 26/-2/-18$; Z-score = 2.44) as well as at the border of the right insula cortex and the rolandic operculum ($x, y, z = 42/2/20$; Z-score = 2.54). Bilateral activation was only found in the putamen ($x, y, z = 22/12/-5$; Z-score = 2.65 and $x, y, z = -22/-12/-2$; Z-score = 2.69). The only left lateralised activation was detected in intraparietal sulcus (IPS) ($x, y, z = -42/-32/44$; Z-score = 2.78).

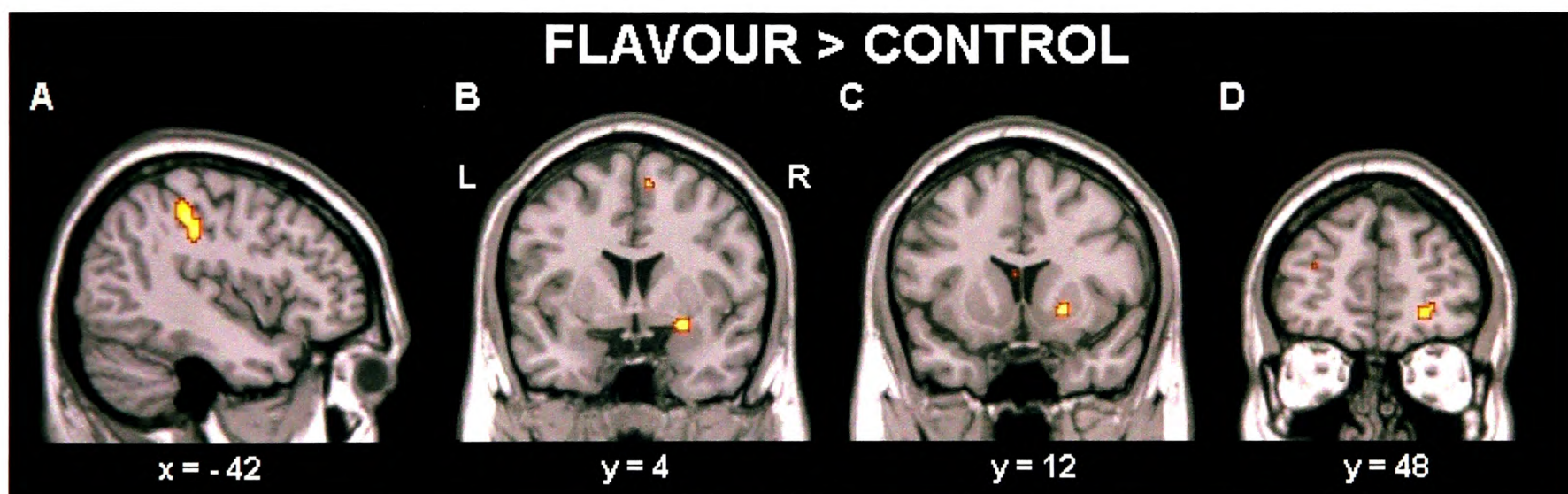


Figure 5.5: Flavour activation for 'hold' experiment.

The activation maps depict the group activation map ($n=20$) of the contrast [FLAVOUR – CONTROL]. The only left lateralised activation was found along the intraparietal sulcus (A). Primary olfactory cortex was only activated on the right side (B), whereas the putamen was activated bilaterally, even though more anterior on the right side (C) than on the left (not shown). OFC activation was found exclusively in the right hemisphere (D).

Since both flavour and control stimuli elicited nearly identical patterns of neural activation, these brain regions appear not to be exclusively activated by the flavour stimulus but rather display higher BOLD signal changes to the flavour

compared to the control condition. To assess the strength of the activation and compare the responses in these apparent flavour-processing areas, the percentage BOLD signal changes were computed for the flavour and control conditions separately (Figure 5.6). The results demonstrate that overall, the BOLD signal changes are in the region of 0.15% with the medial OFC displaying the strongest response in both the flavour and control condition. For the flavour stimulus, all brain regions show a positive BOLD response with the only regions being exclusively activated by the flavour stimulus being located in the right lateral OFC and the putamen. Conversely, the control stimulus elicited positive responses only in medial OFC, piriform cortex and in the IPS, whereas amygdala and insula cortex displayed a negative signal change.

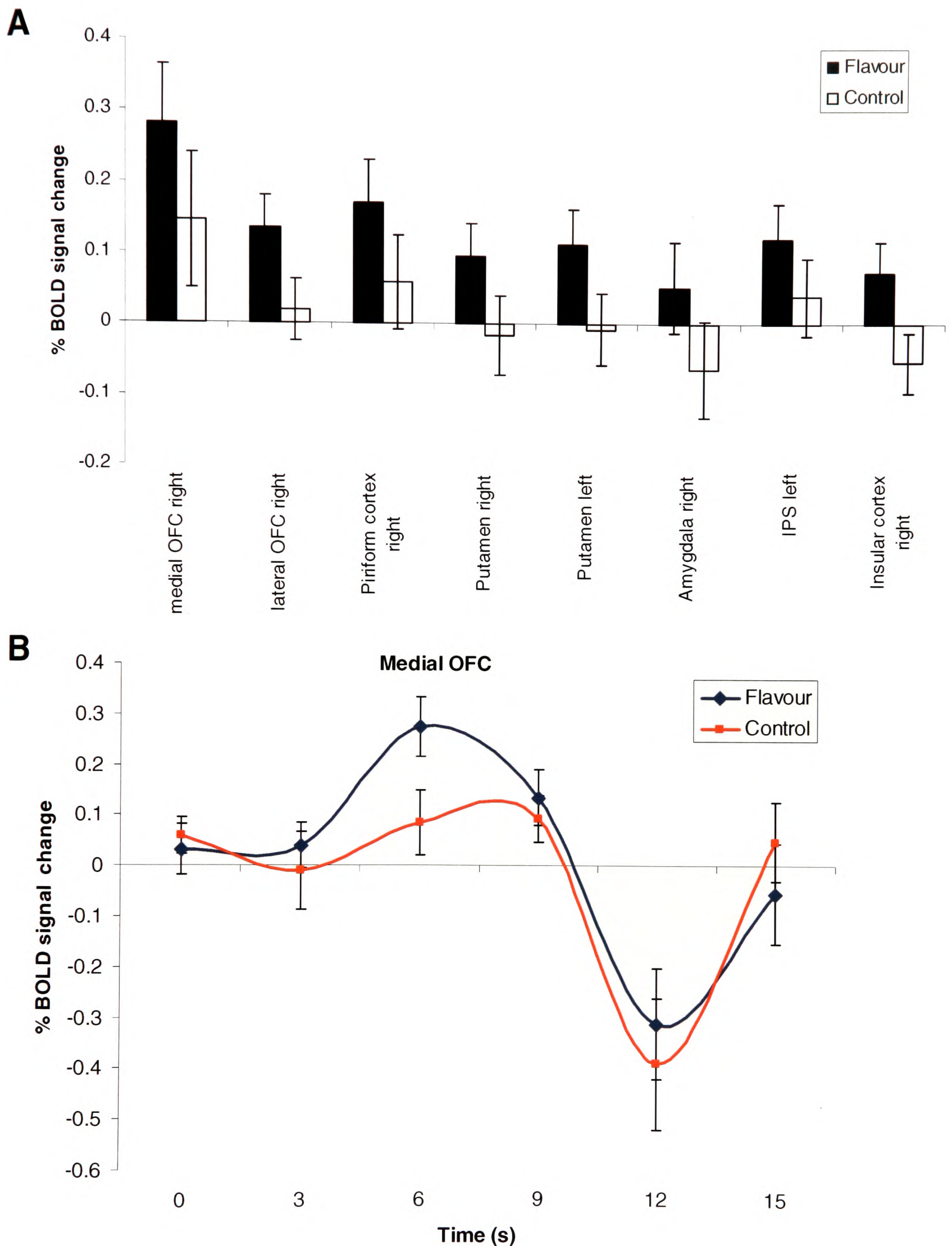


Figure 5.6: BOLD signal changes for ‘hold’ experiment.

The average ($n=20$) percentage BOLD signal changes for all areas emerging from the contrast [FLAVOUR – CONTROL] shown for the flavour and control trials separately (A) as well as the time course for the cluster in the right medial OFC. Error bars depict the standard error.

5.3.3 Swallow experiment

Similar to the findings of the ‘hold’ experiment, when both flavour and control stimuli were compared to the rest periods, the resulting in patterns of activation displayed a high degree of overlap (Figure 5.7) as well as similarly strong responses (Table 5.2). The predominant foci for this activation were located bilaterally along the inferior part of the postcentral sulcus, extending into the rolandic operculum and the insula cortex. Activity likely to be related to the swallowing movement was found in the supplementary motor area (SMA) and the cerebellum.

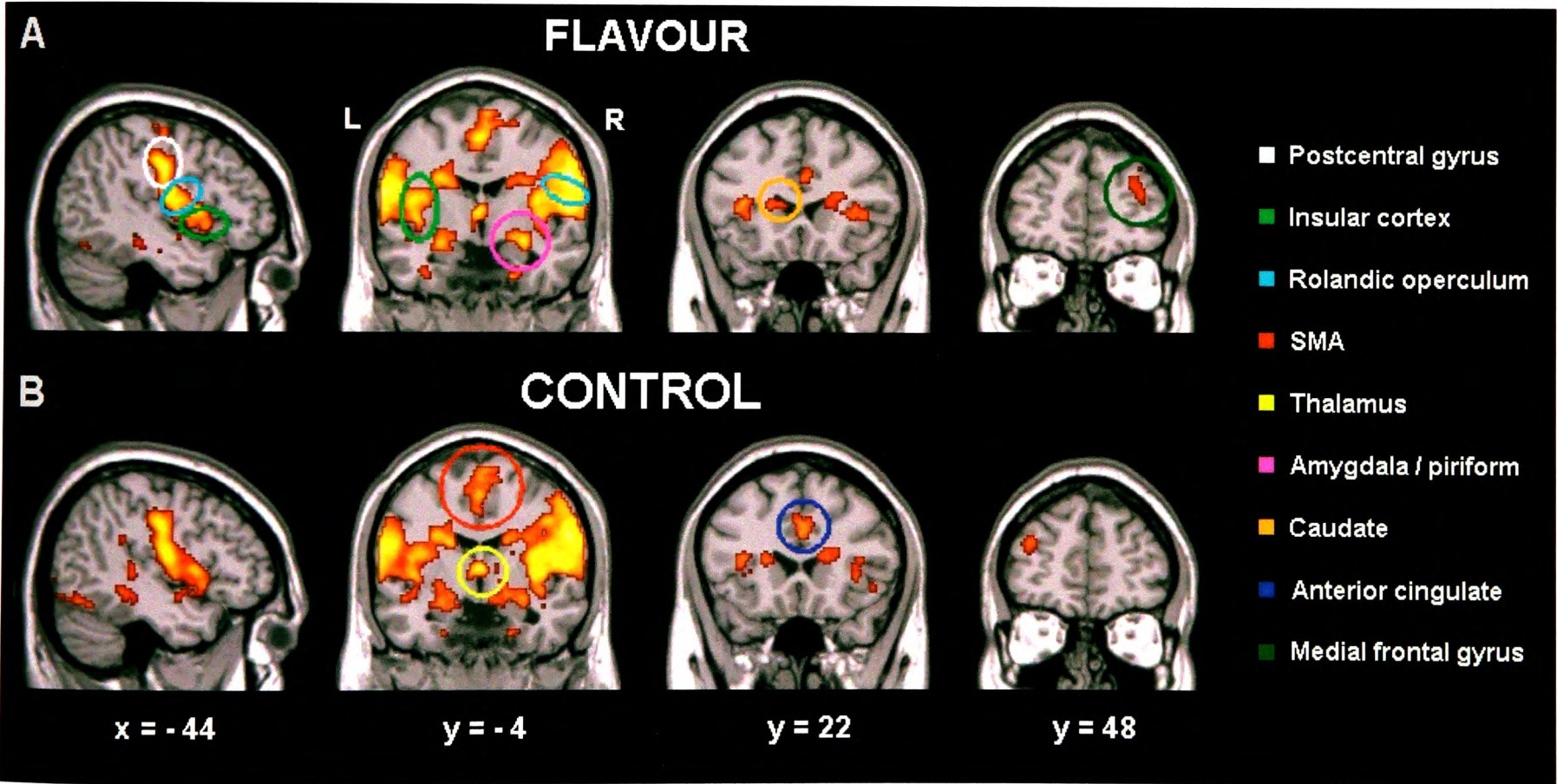


Figure 5.7: Flavour and control activation maps for ‘swallow’ experiment.

Group activation maps (n=20) for both the [FLAVOUR – REST] (A) and [CONTROL – REST] (B) are shown in the canonical MNI 152 standard space. Both types of stimuli resulted in highly similar activation patterns predominantly along the precentral gyrus, frontal and rolandic operculum and insula cortex. The left side of each slice corresponds to the left side of the brain.

Bilateral activation was also detected in limbic structures, namely in the amygdala and adjoining piriform cortex as well as in the caudate and the anterior cingulate. In contrast to the ‘hold’ experiment, no significant activation was observed in the OFC. An interesting lateralisation effect could be observed in the medial frontal gyrus that only activated in the right hemisphere in the flavour condition and in the left hemisphere in the control condition even though in both cases with relatively low z-scores.

Brain region	Side	Flavour		Control	
		MNI coordinates	Z-score	MNI coordinates	Z-score
Anterior insula cortex	R	38/10/6	4.73	42/8/-8	4.52
	L	-42/8/6	4.53	-36/12/4	4.52
Anterior cingulate	R	12/8/36	3.18	4/24/30	3.56
	L	-12/12/32	4.01	-10/12/34	3.83
Medial frontal gyrus	R	34/36/20	2.80	-	-
	L	-	-	-36/48/22	3.11
Caudate	R	20/16/14	4.08	20/18/16	3.86
	L	-18/6/20	4.06	-18/4/20	4.54
Piriform cortex	R	22/2/-12	3.20	22/2/-10	3.05
	L	-22/2/-10	3.16	-20/2/-10	3.86
Amygdala	R	28/-4/-12	4.73	28/-2/-14	3.03
	L	-26/-6/-14	4.63	-22/-2/-14	3.96
SMA	R	2/-2/54	4.98	2/-2/54	4.04
	L	0/-2/52	5.06	0/6/50	4.23
Thalamus	R	2/-8/8	5.13	2/-6/6	5.17
	L	-1/-8/8	5.06	0/-4/6	4.74
Ronaldic operculum	R	58/-2/12	6.16	50/-2/14	5.86
	L	-44/-10/18	5.46	-40/-12/20	5.30
Postcentral gyrus	R	58/-4/28	6.36	58/-4/28	5.88
	L	-48/-12/32	6.18	-52/-10/28	6.41
Cerebellum	R	14/-68/-16	5.89	26/-66/-20	6.10
	L	-12/-70/-18	5.64	-14/-68/-16	5.83

Table 5.2: Localisation of activation for ‘swallow’ experiment.

The group activation shown separately for both flavour and control condition. The coordinate and Z-score relate to the highest activated voxel in each of the clusters in the MNI standard space (n=20).

Since the brain responses described above were evoked similarly by the flavour and control stimulus and are therefore likely to mainly reflect somato-sensory and motor processing, in particular the swallowing movement, the contrast [FLAVOUR – CONTROL] was computed to reveal brain areas which were predominantly involved in the processing of the olfactory and gustatory components of the flavour stimulus.

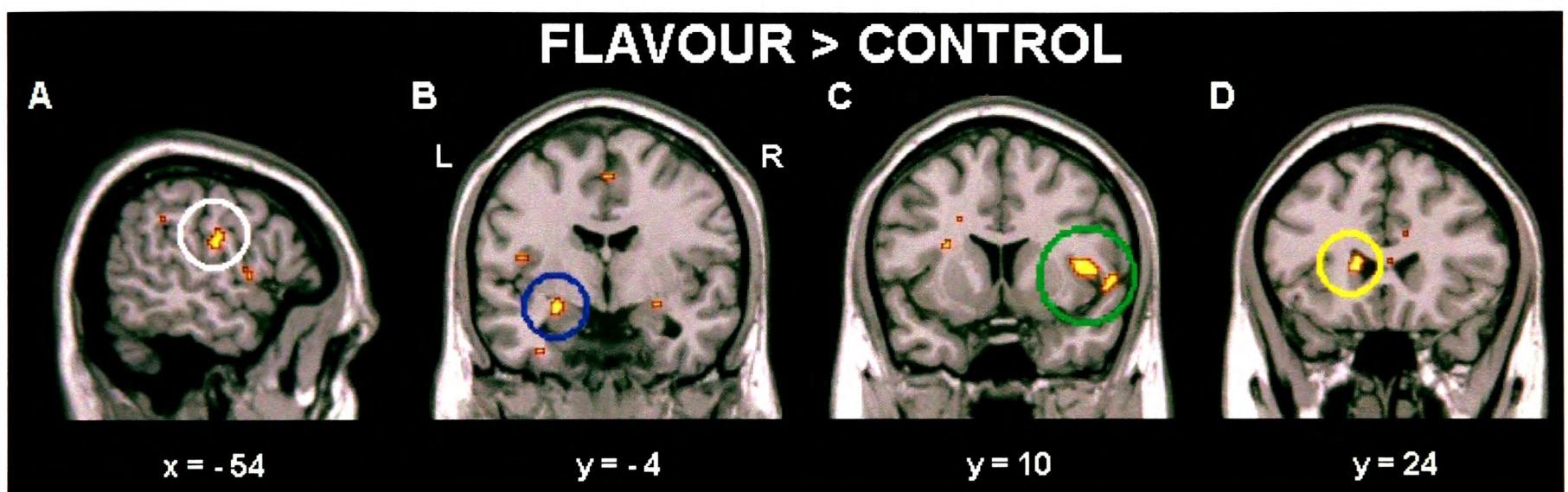


Figure 5.8: Flavour activation for ‘swallow’ experiment.

The activation maps depict the group activation map (n=20) of the contrast [FLAVOUR – Control]. Activation was found bilaterally in the postcentral gyrus (A), the amygdala (B) and anterior insula cortex/frontal operculum (C). Left lateralised activation was observed in the medial caudate nucleus (D).

Activation was restricted to a comparatively small network of areas (Figure 3.5). The strongest effects were found bilaterally in the postcentral gyrus ($x, y, z = 50/-14/24$; Z-score = 2.75 and $x, y, z = -56/-14/24$; Z-score = 2.58) and at the junction of the insula cortex with the frontal operculum, corresponding to the primary taste cortex, even though more anterior in the right hemisphere ($x, y, z = 40/10/8$; Z-score = 2.55) compared to the left ($x, y, z = -42/-2/12$; Z-score = 2.48). Additionally, the

flavour stimulus elicited stronger activation in the left ($x, y, z = -24/-6/-14$; Z-score = 2.94) and right ($x, y, z = 34/-10/-18$; Z-score = 2.30) amygdala compared to the control stimulus. Further differences between the two types of stimuli were detected in the left medial caudate nucleus ($x, y, z = -14/24/8$; Z-score = 2.66). The lateralisation effect observed in the medial frontal gyrus (MFG), where the right hemisphere displayed significant activation only in the taste condition could not be observed in the contrast [FLAVOUR – CONTROL] suggesting that this region displayed activity just below the set statistical threshold in the control condition. A ROI analysis of the relevant cluster in the right MFG confirmed that the percentage BOLD signal changes in this region were nearly identical (0.13 % for the flavour trials and 0.12% for the control trials).

Similar to the findings of the ‘hold’ experiment, the brain regions found active in the contrast [FLAVOUR – CONTROL] are not exclusively activated by the flavour stimulus but rather display higher BOLD signal changes in response to the flavour compared to the control stimulus. To assess the strength of the activation and compare the responses in these apparent flavour-processing areas, the percentage BOLD signal changes were computed for the flavour and control conditions separately (Figure 5.9). The strongest BOLD signal changes were observed bilaterally in the inferior part of the postcentral gyrus. In contrast to the ‘hold’ experiment, where only the right amygdala and insula cortex activated significantly stronger to the flavour than to the control stimulus and also displayed negative signal changes in response to the control stimulus, both areas displayed positive signal changes in the two conditions and in both hemispheres.

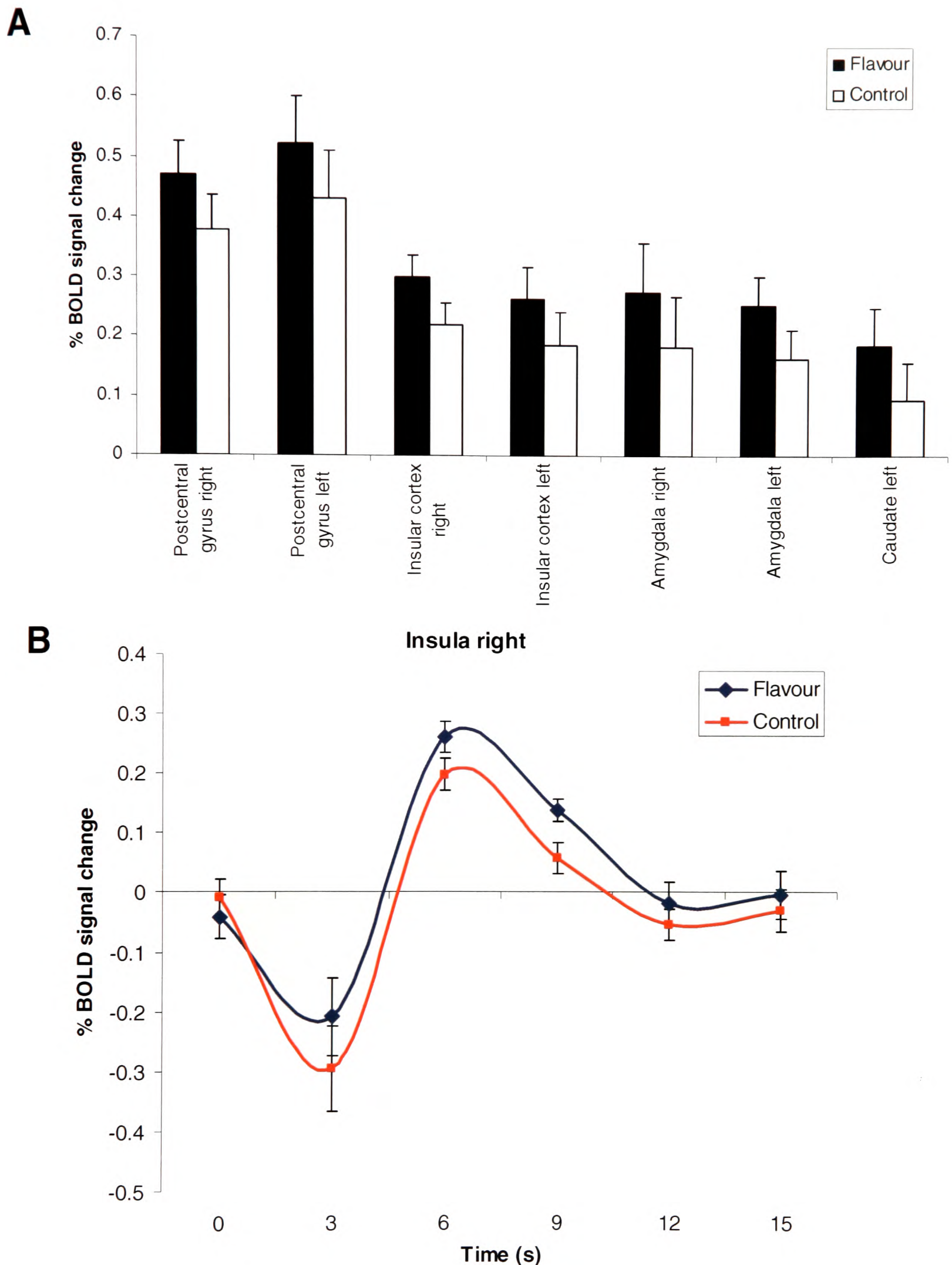


Figure 5.9: BOLD signal changes for ‘swallow’ experiment.

The average ($n=20$) percentage BOLD signal changes for all areas emerging from the contrast [FLAVOUR – CONTROL] shown for the flavour and control trials separately (A) as well as the time course for the cluster in the right insula cortex (B). Error bars depict the standard error.

5.3.4 Activation overlap between ‘hold’ and ‘swallow’ experiments

To compare the activation resulting from the different experimental paradigms, we firstly investigated areas that activated in response to the flavour stimulus in both experiments. Areas of overlapping activation were found bilaterally in the postcentral gyrus extending into the rolandic operculum as well as the frontal operculum (Figure 5.10). Unilateral regions of overlap on the right side were located in the right anterior and posterior insula cortex, the piriform cortex / amygdala, the right medial frontal gyrus and the supramarginal gyrus. The only left lateralised region of overlap was in the left cingulate gyrus (see Table 5.3 for coordinates and z values of the peak voxels in these regions in both experiments).

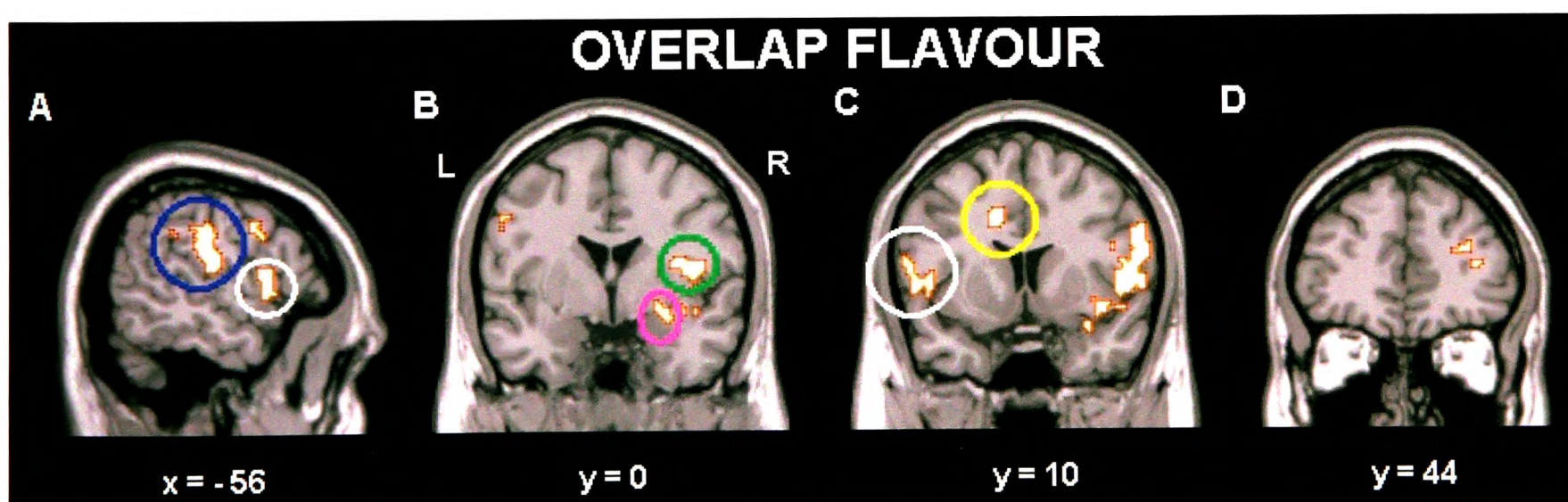


Figure 5.10: Areas of overlap in the two experiments.

Overlapping neural responses to the flavour stimulus in both experiments were found in (A) the postcentral gyrus extending into the rolandic operculum (blue) and the insula cortex extending into the frontal operculum (white) and (B) the right insula cortex (green) and the right piriform cortex / amygdala (pink). (C) Activation in the frontal operculum (white) was more extensive on the right side, whereas a lateralisation to the left hemisphere could be observed in the cingulate gyrus (yellow). The only frontal regions of mutual activation in both experiments were found in the right medial frontal gyrus (D).

Brain region	Side	<i>Hold experiment</i>		<i>Swallow experiment</i>	
		MNI coordinates	Z-score	MNI coordinates	Z-score
Medial frontal gyrus	R	38/42/12	2.94	38/42/12	2.72
Cingulate gyrus	L	-14/8/34	3.25	-12/12/34	3.92
Frontal operculum	R	50/12/4	3.53	60/10/4	5.68
	L	-56/10/2	3.01	-58/6/6	4.78
Anterior insula cortex	R	36/4/-10	3.31	40/4/-8	4.38
Posterior insula cortex	R	42/-2/10	3.05	38/-2/14	4.33
Piriform cortex / Amygdala	R	32/-4/-12	2.85	28/-4/-12	4.73
Postcentral gyrus	R	62/-22/22	5.14	62/-16/18	5.12
	L	-58/-20/18	4.39	-60/-14/20	4.86
Supramarginal gyrus	R	60/-30/36	4.72	52/-36/54	3.91

Table 5.3: Flavour activation overlap between the two experiments.

Mutual regions of activation after flavour stimulation in the two experiments are shown separately for ‘hold’ and ‘swallow’ experiments. The coordinates and Z-scores relate to the highest activated voxel in each of the clusters in the MNI standard space (n=20).

Analysis of the percentage BOLD signal changes (Figure 5.11) in these overlapping regions confirmed that the flavour stimulus elicited positive signal changes ranging from 0.11 to 0.33 percent. However, these regions did not appear to be activated exclusively by the flavour stimulus, since the observed signal changes were nearly identical in response to the control stimulus. On average, activation observed in these overlapping areas was slightly but significantly ($p < 0.05$) stronger in the ‘swallow’ condition (0.21%) than in the ‘hold’ condition (0.17%), an effect that was particularly pronounced along the postcentral gyri.

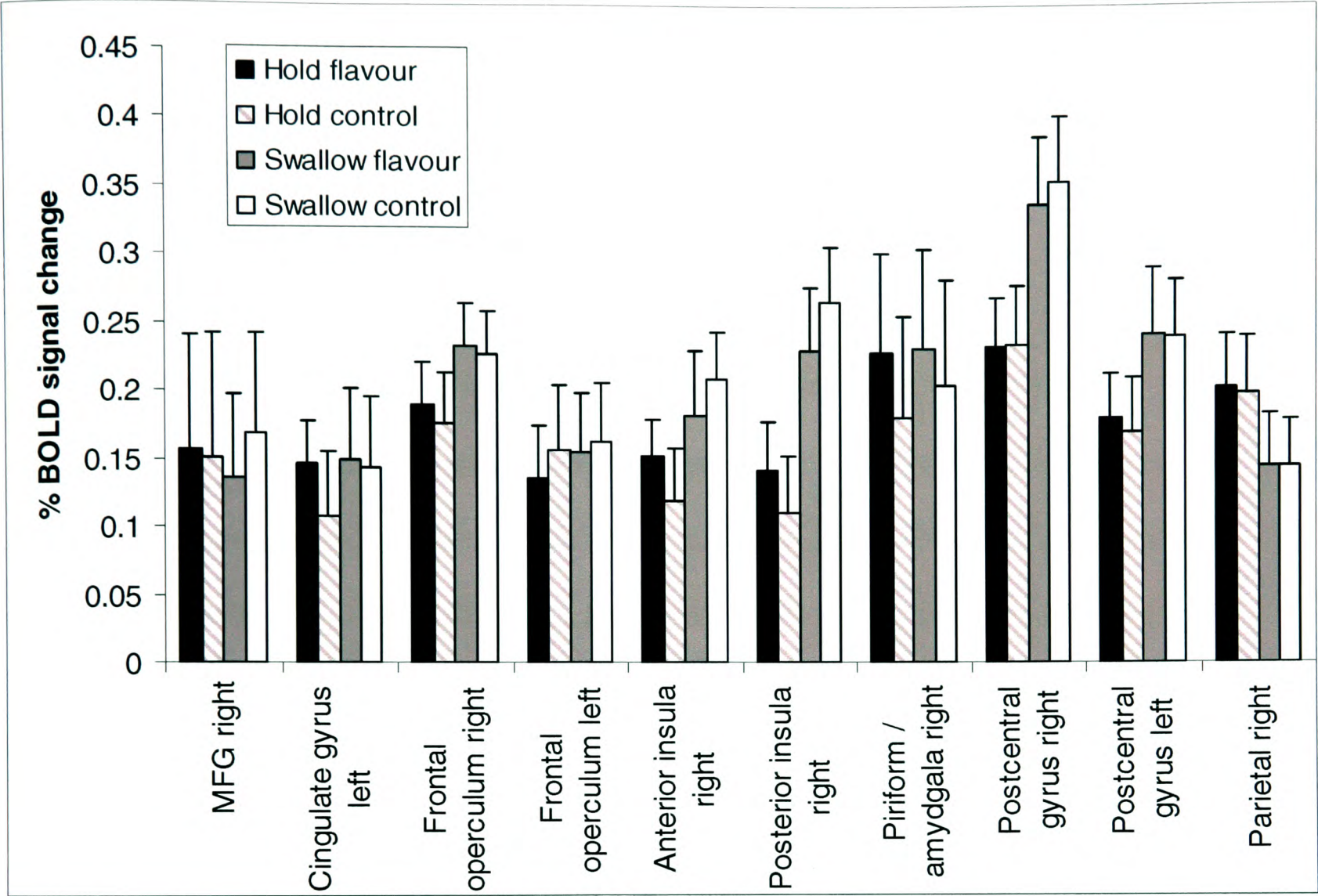


Figure 5.11: BOLD signal changes for regions of overlap.

The average (n=20) percentage BOLD signal changes for all areas being activated by flavour stimulation in both the ‘hold’ and ‘swallow’ experiments shown for the flavour and control trials separately. Error bars depict the standard error.

Surprisingly, when the contrast $[FLAVOUR_{hold} - CONTROL_{hold}]$ was compared with the contrast $[FLAVOUR_{swallow} - CONTROL_{swallow}]$, no areas of overlap were found. This finding may be partly because each of the two contrasts resulted in very small activated clusters. However, both contrasts showed two areas of activation that were in very close proximity, firstly in the left postcentral gyrus (‘hold’ experiment $x, y, z = -42/-32/44$; Z-score = 2.78 and ‘swallow’ experiment $x, y, z = -$

36/-28/44; Z-score = 2.63) and secondly in the right amygdala ('hold' experiment $x, y, z = 28/-4/-12$; Z-score = 2.78 and 'swallow' experiment $x, y, z = 34/-10/-18$; Z-score = 2.30).

5.3.5 Activation differences 'hold' vs. 'swallow' experiments

In order to investigate differences in neural activation between the two experimental paradigms, the contrast [FLAVOUR_{hold} – FLAVOUR_{swallow}] as well as [FLAVOUR_{swallow} – FLAVOUR_{hold}] were calculated. Since these contrasts both contained the flavour trials, the resulting contrasts should highlight areas that were particularly sensitive to the mode of stimulus delivery. The results showed that frontal and orbitofrontal brain regions were activated by the flavour stimulus in the 'hold' experiments only (Figure 3.12 D), with bilateral activation in the lateral orbital gyrus ($x, y, z = 32/34/-18$; Z-score = 4.46 and $x, y, z = -42/30/-18$; Z-score = 4.37) and a large cluster of activation along the medial wall of the gyrus rectus extending into both hemispheres ($x, y, z = -24/-6/-14$; Z-score = 6.01). Additionally, the inferior frontal gyrus displayed stronger activation on the left side only ($x, y, z = -38/12/32$; Z-score = 4.53). Within the temporal lobes, the medial temporal gyri ($x, y, z = 60/-2/-24$; Z-score = 4.68 and $x, y, z = -64/-22/-22$; Z-score = 4.24) and within the parietal lobes, the angular gyri ($x, y, z = 44/-72/44$; Z-score = 4.34 and $x, y, z = -46/-68/-32$; Z-score = 4.75) activated significantly stronger bilaterally during the 'hold' experiment. Interestingly, all regions reported above displayed positive BOLD signal changes in the 'hold' experiment, but negative ones in the 'swallow' experiment (Figure 5.13). Additionally, it should be noted that even though activation in these regions differed

according to the experimental paradigm, the BOLD signal changes were virtually identical for flavour and control stimuli.

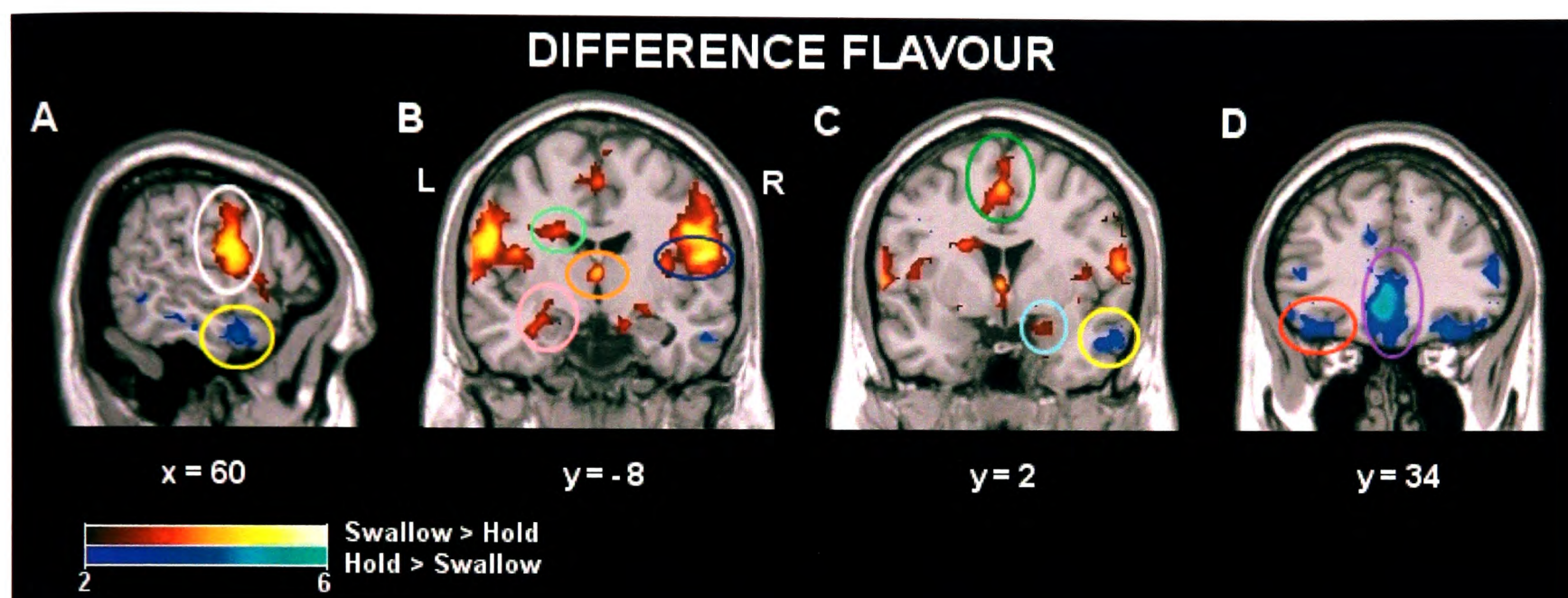


Figure 5.12: Differences between the flavour trials.

Group activation maps ($n=20$) for the contrasts [FLAVOUR_{swallow} – FLAVOUR_{hold}] and [FLAVOUR_{hold} – FLAVOUR_{swallow}] are displayed by the red to yellow and blue to green colour scheme respectively.

Activation was significantly stronger in the 'hold' experiment mainly in the medial (D; purple) and lateral (D; red) OFC and bilaterally in the anterior medial temporal gyrus (A; yellow).

Activation was significantly stronger in the 'swallow' experiment along the postcentral gyrus (A; white) extending into the rolandic operculum (B; blue). Activation was also stronger bilaterally in the amygdala (B; pink), the medial thalamus (B; orange) and the SMA (C, green). Lateralised activation was observed in the right piriform cortex (C; turquoise) and left caudate (B; green).

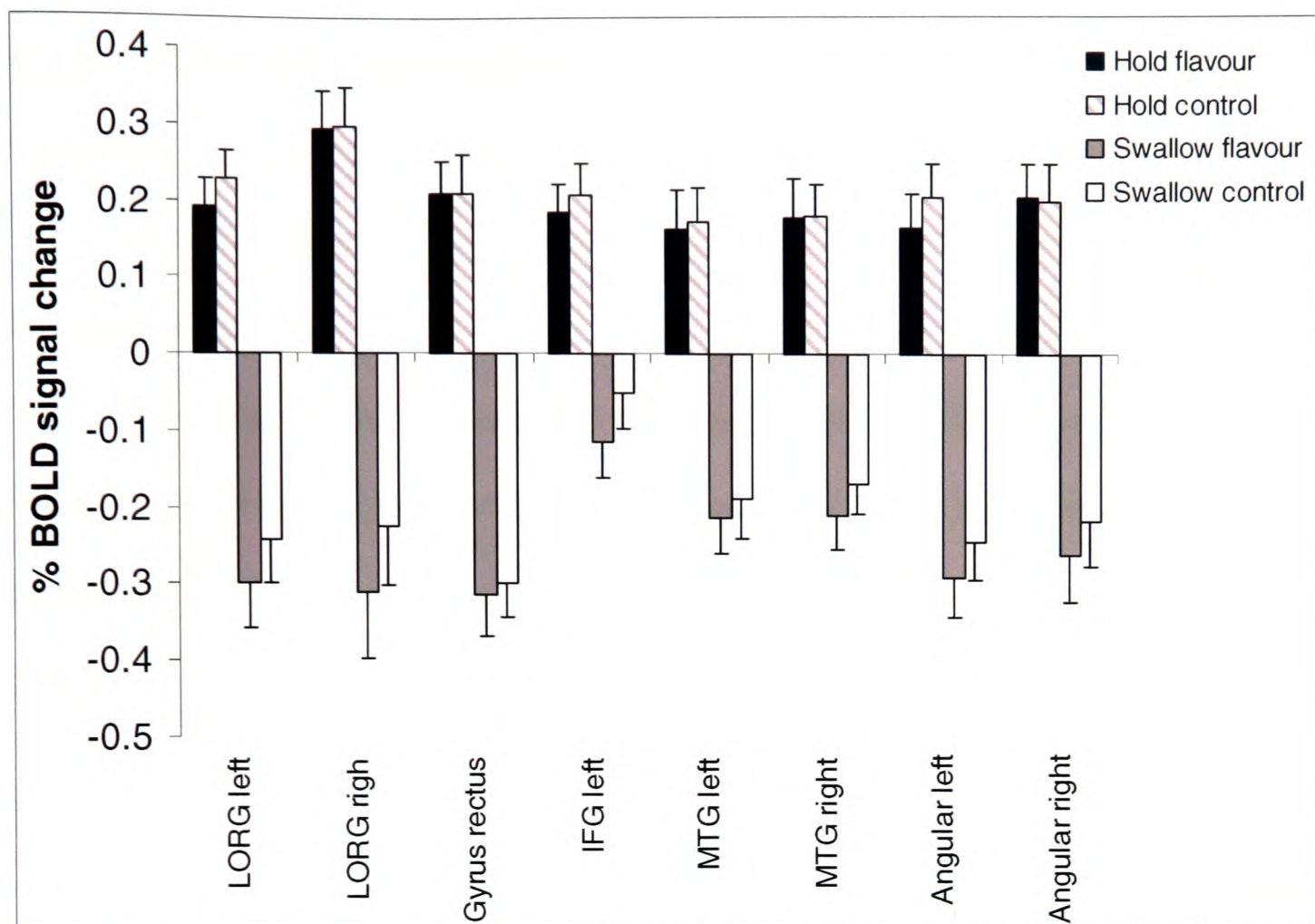


Figure 5.13: BOLD signal changes for [FLAVOUR_{hold} – FLAVOUR_{swallow}].

The average (n=20) percentage BOLD signal changes for all areas showing significant activation in the contrast [FLAVOUR_{hold} – FLAVOUR_{swallow}]. The signal changes are shown for the flavour and control trials in each experimental condition separately. Error bars depict the standard error.

Conversely, for the ‘swallow’ experiment significantly stronger activation could be observed bilaterally along the postcentral gyrus ($x, y, z = 50/-8/28$; Z-score = 4.93 and $x, y, z = -46/-14/34$; Z-score = 4.99) extending into the rolandic operculum ($x, y, z = 56/-10/18$; Z-score = 5.15 and $x, y, z = -40/-10/18$; Z-score = 4.35) as well as in the anterior insula cortex ($x, y, z = 44/6/8$; Z-score = 3.89 and $x, y, z = -42/8/4$; Z-score = 3.51) and the cerebellum ($x, y, z = 6/-74/-12$; Z-score = 5.08 and $x, y, z = -4/-80/-16$; Z-score = 4.78). Activation differences were also detected in the medial thalamus ($x, y, z = 2/-6/10$; Z-score = 4.88), the SMA ($x, y, z = 2/0/52$; Z-score = 4.57) and the amygdala ($x, y, z = 26/-10/-10$; Z-score = 3.28 and $x, y, z = -32/-8/-22$; Z-score

= 3.49). Additionally, lateralised activation was found in the right piriform cortex ($x, y, z = 24/6/-22$; Z-score = 3.39) and left caudate ($x, y, z = -18/6/22$; Z-score = 3.79).

Analysis of the BOLD signal changes in these regions again showed that the activation in these regions was nearly identical for the flavour and control trials (Figure 5.14). Interestingly, the regions that were most strongly activated in the ‘swallow’ experiment (postcentral gyrus, rolandic operculum and anterior insula cortex) did not display any significant activation during the ‘hold’ experiment and all other areas showed a negative BOLD response.

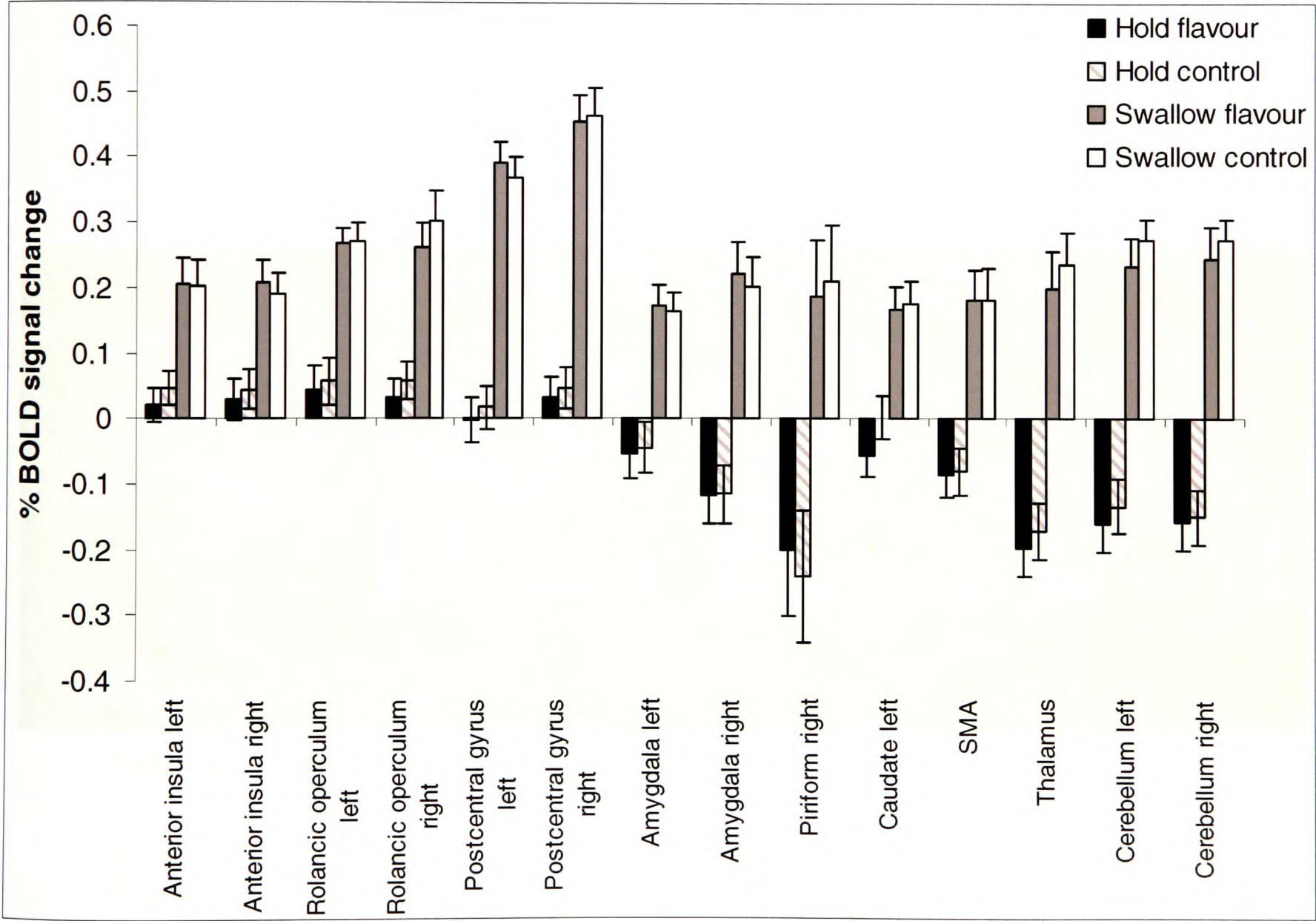


Figure 5.14: BOLD signal changes for [FLAVOUR_{swallow} – FLAVOUR_{hold}].

The average (n=20) percentage BOLD signal changes for all areas showing significant activation in the contrast [FLAVOUR_{swallow} – FLAVOUR_{hold}]. The signal changes are shown for the flavour and control trials in each experimental condition separately. Error bars depict the standard error.

Finally a comparison was made between the contrasts [FLAVOUR – CONTROL] in the two experimental conditions to investigate if the two modes of flavour stimulation would engage gustatory and olfactory pathways in a different manner, independent of somato-sensory or motor activity. As noted earlier, both flavour and control stimuli elicited nearly identical patterns of activation with only very circumscribed regions displaying stronger responses to the flavour stimulus. Consequently, the contrast [FLAVOUR_{hold} – CONTROL_{hold}] – [FLAVOUR_{swallow} – CONTROL_{swallow}] and the contrast [FLAVOUR_{swallow} – CONTROL_{swallow}] – [FLAVOUR_{hold} – CONTROL_{hold}] resulted in very small clusters of activation (Figure 5.15).

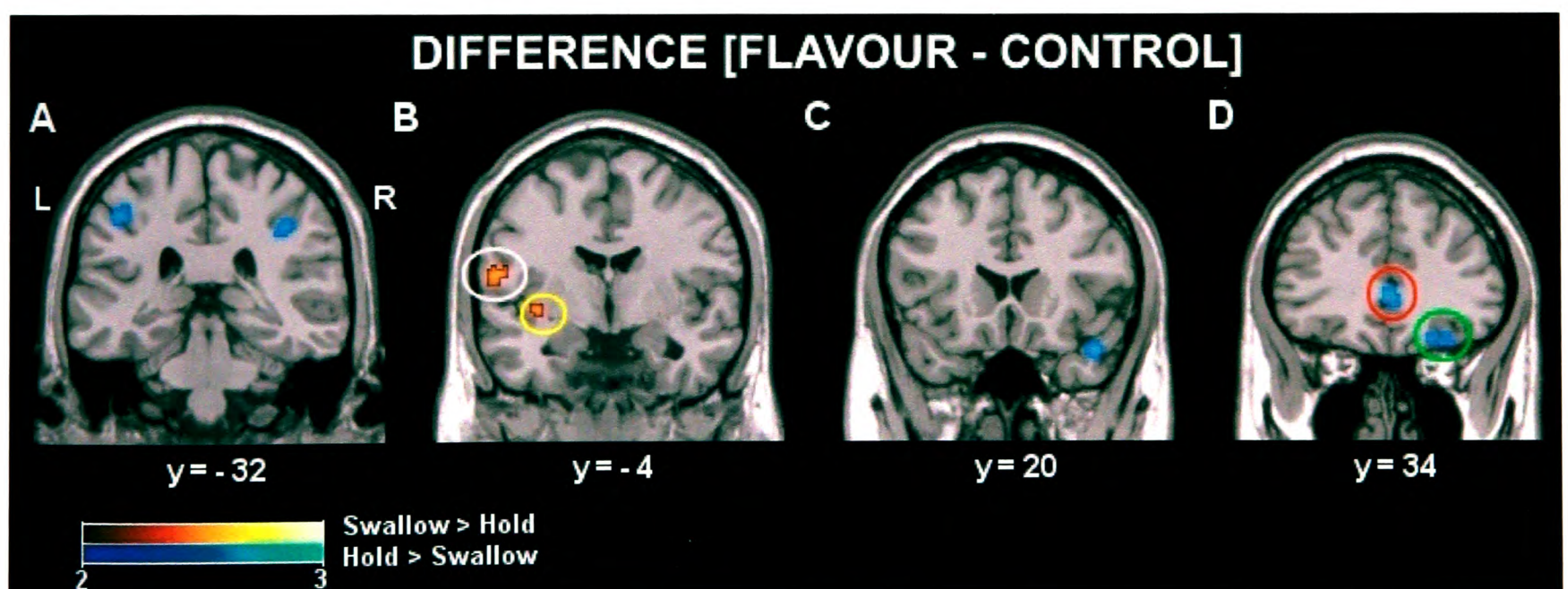


Figure 5.15: Differences in flavour activation.

Group activation maps (n=20) for the comparison between the contrasts [FLAVOUR_{swallow} - CONTROL_{swallow}] and [FLAVOUR_{hold} - CONTROL_{hold}] which are displayed by the red to yellow and blue to green colour scheme respectively.

Activation was significantly stronger in the 'hold' experiment within the IPS in both hemispheres (A) and lateralised to the right hemisphere in the temporal pole (C) and OFC (D; green). Additional activation differences were found in the anterior cingulate gyrus (D; red). The 'swallow' experiment elicited stronger activation in left opercular (B; white) and insula (B; yellow) regions only.

Several frontal brain regions in the right hemisphere displayed stronger differences between flavour and control trials in the 'hold' experiment compared to the 'swallow' experiment, namely the right medial ($x, y, z = 24/34/-20$; Z-score = 2.61) and lateral ($x, y, z = 30/44/-4$; Z-score = 2.64) OFC and the anterior cingulate gyrus ($x, y, z = 2/34/4$; Z-score = 2.71). Additional lateralised activation was found in the right temporal pole ($x, y, z = 46/20/-24$; Z-score = 2.92) and the left putamen ($x, y, z = -24/-10/2$; Z-score = 2.91) whereas the only bilateral activation was located in the IPS ($x, y, z = 40/-32/40$; Z-score = 2.71 and $x, y, z = -44/-32/44$; Z-score = 2.88).

Conversely, stronger activation during the 'swallow' experiment was restricted to the rolandic operculum ($x, y, z = -56/-4/14$; Z-score = 2.80) as well as the middle ($x, y, z = -36/-4/-4$; Z-score = 2.76) and posterior ($x, y, z = -44/-18/2$; Z-score = 2.86) insula cortex in the left hemisphere only.

Analysis of the BOLD signal changes (Figure 5.16) demonstrated again that the majority of these regions are not exclusively activated by the flavour stimulus but also respond to the control stimulus, even though with slightly lower amplitudes. Similar to the comparisons of the flavour trials only between the two experiments (as described above) the swallow experiment elicited negative BOLD signal changes in orbitofrontal regions, the anterior cingulate and the temporal pole. The only significant negative BOLD changes for the 'hold' experiment were observed in the left posterior insula cortex.

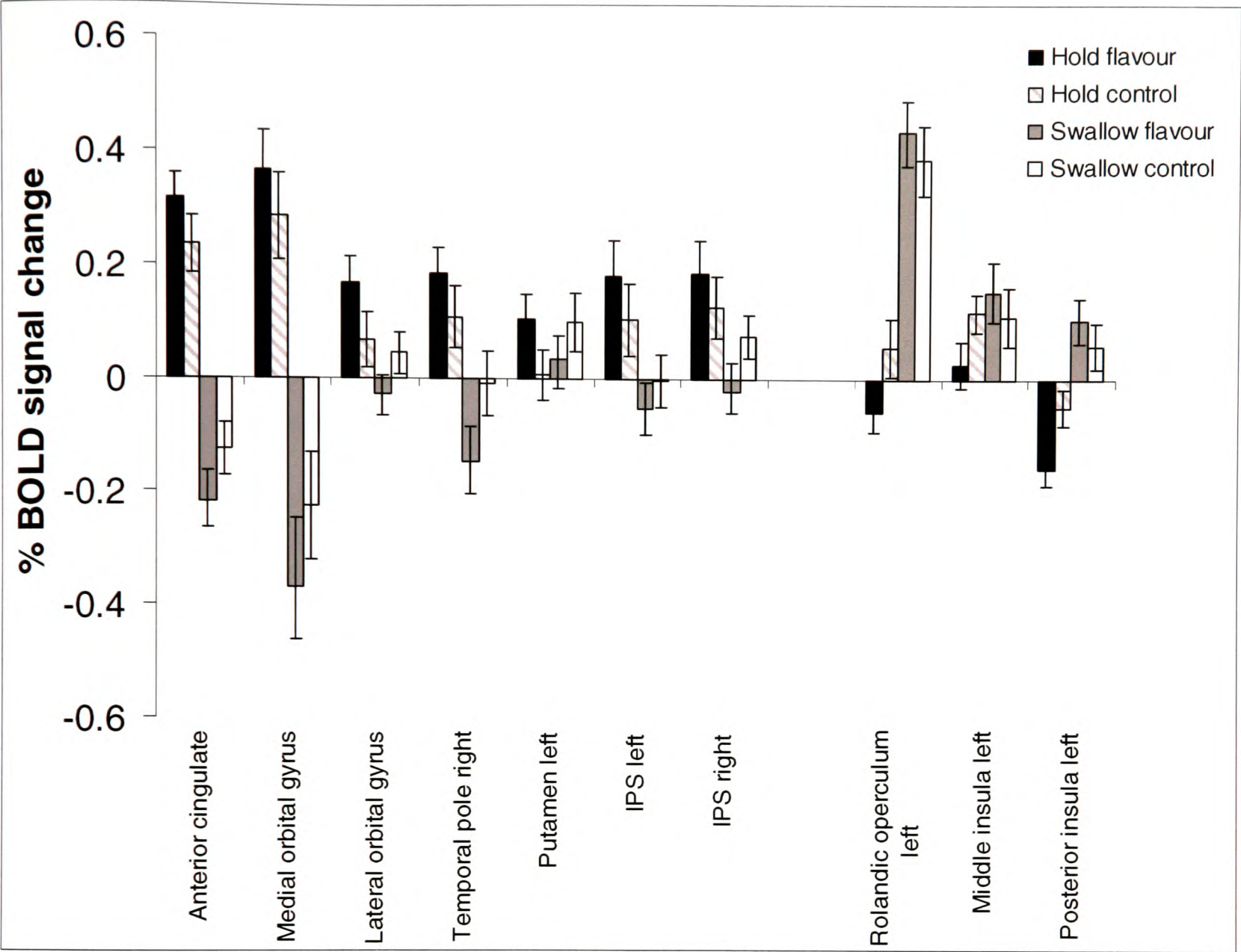


Figure 5.16: BOLD signal changes for [FLAVOUR – CONTROL].

The average (n=20) percentage BOLD signal changes for all areas showing differences between ‘hold’ and ‘swallow’ experiments in the contrast [FLAVOUR– CONTROL]. The signal changes are shown for the flavour and control trials in each experimental condition separately. Error bars depict the standard error.

5.4 DISCUSSION

The aim of this set of experiments was to investigate similarities and differences between two different modes of flavour stimulation in the fMRI environment. More specifically, I was interested to determine whether it was possible to obtain data of sufficient quality when subjects were instructed to swallow a flavour stimulus directly after its delivery, compared to the ‘traditional’ method of holding the stimulus in their mouth for a period of time and only swallowing after the relevant activation data was collected. A particular concern with the ‘traditional’ method was that holding the stimulus in the mouth whilst in the supine position would not permit sufficient retronasal olfactory stimulation. Consequently, results might be biased towards the gustatory modality. Conversely, swallowing the stimulus was expected to elicit stronger olfactory activation. However, using this method, stimulus-correlated head movements could have introduced further motion artefacts.

Results from the head motion analysis showed that over the course of the entire experiment, motion was virtually identical in the two experimental conditions. This is unsurprising given that swallowing movements were equated in both conditions. However, there were crucial differences in the time intervals when these head movements occurred. Whereas head motion was significantly stronger during the stimulation periods in the ‘swallow’ experiments compared to the resting periods, the opposite was found for the ‘hold’ experiment. This is a strong indication that the majority of head motion in experiments using liquid stimuli is caused by swallowing. Therefore the potential benefit of increased olfactory stimulation associated with swallowing the stimulus comes at the cost of increased head motion that is also correlated with stimulus perception. Even though no strong motion artefacts were observed in the dataset, this increased motion may cause false positives in the

functional analysis. To a certain extent this problem is reduced by subtracting the control condition from the flavour condition, assuming the amount of motion does not differ between the two.

As previously discussed (Chapter 3), the choice of distilled water mixed with KCl and NaHCO₃ in concentrations presumed to be similar to those found in human saliva as the control solution - as suggested by Rolls and colleagues (de Araujo et al. 2003a; O'Doherty et al. 2001), was somewhat problematic. Participants reported that this liquid had a particular taste that was similar to water, albeit slightly salty. If correct, this would likely explain why the control solution elicited a very similar pattern of functional activation to that elicited by the flavour stimuli in both experiments, since it would engage gustatory brain areas. Therefore, it may be preferable in future studies to measure the relevant ionic components in the saliva of each participant and calibrate the control solution accordingly. Alternatively, several control solutions spanning a range of ionic concentrations of these relevant components could be tested in each participant prior to scanning and the one perceived as most 'neutral' on an individual basis would then be utilised as the control solution for a subsequent fMRI investigation. Finally, an area for future research must be to determine whether such an apparently 'tasteless' liquid (with a viscosity equal to water) delivered at room temperature is necessarily the best choice for a control solution, or whether it would be more appropriate to use a liquid with a viscosity and temperature (and hence 'mouth-feel') more closely resembling that of human saliva.

To illustrate the effect of the control solution on cerebral activation, activation maps for the control solution were generated for both experimental sessions. Results from the 'hold' experiment showed that both control and flavour stimuli elicited similar activation patterns in brain regions associated with gustation and olfaction,

such as insula cortex and adjoining frontal operculum, OFC, and anterior cingulate (de Araujo et al. 2003c; Frank et al. 2003; Kringelbach et al. 2003; O'Doherty et al. 2001; Rolls 2004c; Schoenfeld et al. 2004; Small et al. 2003; Small et al. 2004b). The only brain region activated exclusively by the flavour stimulus was the right piriform region corresponding to primary olfactory cortex (Poellinger et al. 2001; Zelano et al. 2005), and adjoining amygdala. Such activation would be predicted in view of the olfactory component present in the flavour solution only.

However, for the contrast [FLAVOUR – CONTROL], relatively few clusters were found to respond more strongly to the flavour solution. These were predominantly right-hemispheric, supporting claims that basic processing of odours is lateralised to the right side of the brain (Brand et al. 2001; Zatorre et al. 1992). The flavour stimulus was found to elicit stronger activation in right piriform and medial and lateral OFC, corresponding to primary and secondary olfactory cortices respectively (Rolls 2004c; Small et al. 2004b), as well as in the primary taste cortex located in the insula (Ogawa et al. 2005; Small et al. 1999a). Additionally, the flavour stimulus also activated the putamen, a region previously implicated in coding the reward value of flavour stimuli and food preferences (O'Doherty et al. 2006). Although no behavioural measure of the perceived pleasantness of the two stimuli was acquired during scanning, this finding suggests that the participants preferred the flavoured solution over the control solution.

Finally, left-lateralised activation was observed in the intraparietal sulcus (IPS), a polymodal region previously implicated in multisensory integration of audio-visual (Calvert et al. 2001), visual-tactile (Peltier et al. 2006) and visual-olfactory (Gottfried and Dolan 2003) inputs. Even though direct inputs from piriform cortex to IPS have so far not been demonstrated, the IPS receives olfactory projections via the

OFC and amygdala (Carmichael and Price 1995b; Cavada and Goldman-Rakic 1989). Since the IPS is thought to play a mayor role in the spatial integration of sensory cues, a potential role during olfactory-visual integration in IPS might be to enhance the odour source localization or to help orient attention toward a particular odour. The notion that spatial representations of odours might depend on the functioning of this region is further substantiated by the observation that olfactory neglect occurs in patients with right parietal lobe damage (Bellas et al. 1988). With respect to the multisensory integration between the chemical senses, the IPS may be crucially involved in the localisation of the odour to the mouth rather than the surrounding air. Additionally, superadditive responses have previously been reported in IPS for congruent odour-taste combinations (Small et al. 2004b), which further implicates the IPS as a key region in multisensory processing including the chemical senses.

As with the ‘hold’ experiment, activation in the ‘swallow’ experiment displayed a high degree of overlap between the flavour and control conditions. Both types of stimuli elicited strong responses in the inferior part of the postcentral sulcus, supplementary motor area and cerebellum, regions previously found activated during tongue movements (Fesl et al. 2003; Martin et al. 2004) as well as automatic and volitional swallowing (Furlong et al. 2004; Martin et al. 2001; Toogood et al. 2005). Activation likely to be caused by chemosensation was found in the piriform cortex/amygdala, the anterior insula cortex, medial aspects of the thalamus and the anterior cingulate.

Surprisingly, neither of the two stimuli elicited activation in the OFC. This is a grave concern for studies involving flavour perception since this brain region has been consistently reported as being crucially involved in the integration of the chemical senses and hence the formation of the flavour percept (de Araujo et al. 2003, Small et

al. 2004). This is also a somewhat unexpected finding since the increased olfactory stimulation induced by swallowing the liquid was hypothesised to lead to increased activation not only in primary olfactory cortex (which it did) but also in secondary olfactory regions such as the OFC. It is therefore highly likely that the absence of OFC activation is predominantly due to the increased head motion induced by the swallowing movements (Elliott et al. 2004; Hutton et al. 2002) which lead to increased physiological noise in this region and in turn reduced signal detectability.

As expected, subtracting the control condition from the flavour condition eliminated most activation in somatosensory and motor regions. The flavour stimulus lead to significantly stronger bilateral activation in primary gustatory and olfactory cortex located in the anterior insula and piriform cortex/amygdala as previously shown by others (de Araujo et al. 2003c; Marciani et al. 2006; Ogawa et al. 2005; Small et al. 1997; Small et al. 2004b). However, the flavour stimulus also appeared to engage a region in the postcentral gyrus previously identified as being involved somatosensory processing of the tongue area (Hesselmann et al. 2004; Miyamoto et al. 2006). Since it can not be ruled out that participants performed more tongue movements in the presence of the flavour stimulus compared to the control solution, this result does not necessarily imply that this region is involved in chemosensation.

When comparing the mutual regions of activation after application of the flavour stimulus between the two experimental paradigms (e.g. $\text{FLAVOUR}_{\text{hold}} \cap \text{FLAVOUR}_{\text{swallow}}$), it was found that both activated primary chemosensory cortices as well as the postcentral and cingulate gyrus. When comparing the percentage signal changes in these regions, slightly (but significantly) stronger activation was observed for the ‘swallowing’ experiment. This suggests that the more ‘natural’ stimulus delivery method in this condition and the resulting heightened olfactory component

may have lead to increased activation in flavour processing areas. However, following subtraction of the control condition from the flavour condition in both experiments, no areas of overlap were found. This may be due in part to the small cluster sizes found in both conditions.

Even though no overlapping areas could be detected, adjacent clusters in the left postcentral gyrus and right amygdala were observed, indicating that both regions are similarly engaged by both experimental paradigms. On the other hand, the absence of overlapping activation may indicate that both experimental conditions result in a different neuronal representation of the flavour stimulus and as a result a slightly different flavour percept. To further investigate this possibility, the differences between the two conditions were calculated. When comparing the responses to the flavour stimulus in both experiments (without subtraction of the control), it was found that the ‘hold’ condition predominantly activates OFC. A detailed analysis of the associated BOLD signal changes demonstrated strong negative signal changes in the ‘swallow’ condition. Even though effects of response depression have previously been reported for flavour stimuli (Small et al. 1997) the drop in signals originating from OFC regions are likely to be caused by head motion due to swallowing, as detailed above. Conversely, swallowing the stimulus increased activation predominantly in the postcentral gyrus and adjoining rolandic operculum, both of which are likely to mainly reflect sensory-motor activation (Fesl et al. 2003; Furlong et al. 2004; Gow et al. 2004; Hesselmann et al. 2004; Miyamoto et al. 2006). Importantly, the comparison between the flavour trials in the two experimental conditions revealed that swallowing engaged primary gustatory (anterior insula/operculum) and olfactory (piriform/amygdala) cortices as well as putative flavour processing areas in the anterior insula more strongly than passively holding the stimulus in the mouth. This is

expected, since it is well known that multisensory binding depends to a large extent on the temporal synchronicity of stimuli (Stein, 1998). The concurrent olfactory, gustatory and somatosensory stimulation experienced during swallowing of the stimulus is therefore likely to have facilitated the multisensory binding process, resulting in enhanced activation in flavour regions such as anterior insula and amygdala. Therefore, this finding highlights the importance of retronasal olfactory stimulation resulting from swallowing the liquid in the generation of the flavour percept. Additionally, this may suggest that the act of swallowing a liquid present in the mouth draws attention to chemosensation and engages primary sensory cortices in a similar manner to that observed during sniffing (Mainland and Sobel 2006; Sobel et al. 1998a). Consequently, the act of swallowing may be regarded as constituting an integral part in the formation of the flavour percept, since the temporal and spatial contiguity induced by it are crucial determinants of odour/taste integration because they promote the perception of disparate taste, odour and oral somatosensations as a common object, namely a food/drink (Small & Prescott, 2005).

Finally, a comparison was made between the activation elicited exclusively by the flavour stimulus (i.e. the contrast [FLAVOUR – CONTROL]) during the two experimental sessions. Holding the stimulus in the mouth resulted in stronger activation in the medial and lateral OFC, anterior cingulate, temporal pole, putamen and IPS, all of which have previously been implicated in flavour processing (de Araujo et al. 2003c; Kringelbach et al. 2003; Small et al. 1997; Small et al. 2004b; Small et al. 1999a). Since these regions were also activated to a lesser degree by application of the control solution it appears that at least part of the neuronal population contained in these regions is involved in somatosensory and processing of the tongue area and oral cavity, such as ‘mouth-feel’ (temperature and viscosity) of

the liquid as well as the tongue movements performed during ‘tasting’ of the stimuli. Conversely, swallowing the stimulus immediately after delivery resulted in significantly stronger activation only in the left insula cortex and adjoining opercular regions. Whilst these brain regions do contain the primary gustatory cortex (Rolls 2006), parts of these regions are involved in flavour processing which includes representations of the oral somatosensory properties of stimuli, such as viscosity, fat texture, temperature and capsaicin (De Araujo and Rolls 2004; Verhagen et al. 2004). Therefore, swallowing of the stimulus may lead to a stronger engagement of these multisensory flavour areas, compared to passively holding it in the mouth.

To conclude, the results of this study suggest that the control liquid has to be carefully chosen and that activation resulting after application of the control stimulus should be explicitly modelled, rather than being simply included in the rest period. The observation that the control liquid caused at least some activation in flavour processing areas may also explain why most neuroimaging studies do not observe effects without the use of region of interest (ROI) analysis, which allows for a lowered threshold to detect activation differences (de Araujo et al. 2003c; Small et al. 2004b). Furthermore, the results of this study demonstrate that it is feasible to have participants swallow a liquid whilst collecting the haemodynamic responses, thereby allowing for increased retronasal olfactory stimulation as well as a more ‘natural’ way of perceiving flavours. However, the increased head motion associated with this may potentially mask activity particularly in OFC areas, an effect that may be minimised by incorporating a bite-bar into the experimental set-up.

6 Colour-odour associations

Colours have been demonstrated to influence odour perception in humans. Congruently coloured odorous solutions (e.g., a green coloured mint solution) have been reported to increase the subjective perception of the intensity and pleasantness of the odour compared to incongruent or colourless solutions. Here, I used a sensory colour-odour matching task to investigate whether certain familiar odours would be matched to their naturally associated colour. Additionally, I tested which colours people judge to be incongruent with a given odour. The intensity, pleasantness and familiarity of each odour as well as its identity were also assessed. The results show that participants consistently and reliably match odours with strong colour associations (e.g., lemon and yellow) and that grey is most frequently chosen as an incongruent 'colour'. Additionally, while odours were generally judged as familiar, this did not equate with high identification ability. The results of this study also informed the selection of odour stimuli for the fMRI study in Chapter 7.

6.1 INTRODUCTION

There has been a recent increase of interest in studying interactions between different sensory systems in humans and other species (Calvert et al. 2004; Marcs 1978; Stein and Meredith 1993) including intersensory interactions involving the chemical senses (Gottfried and Dolan 2003; Prescott et al. 2004; Small 2004; Stillman 2002). Importantly, in our natural surroundings, odours are seldom perceived in

isolation. Rather, odours are often accompanied by visual cues that can modulate our subjective experience of the stimuli from which they originate. So far, such visual influences have been most extensively studied in the context of food and flavour perception (Clydesdale 1993; King and Duineveld 1998; Kostyla and Clydesdale 1978).

Our perception of the foods and beverages we consume typically involves the integration of multiple sensory inputs relating to the taste, smell, look, texture, temperature and trigeminal attributes of a product (de Wijk et al. 2004; Engelen et al. 2003; Rolls 2004c; Small 2004; Stillman 2002; Yackinous and Guinard 2001; Zampini and Spence 2004). Together, these multisensory interactions play a critical role in delivering a unified flavour percept and also greatly influence the perceived pleasantness of the foodstuffs we consume.

Colour in particular has been shown to have a substantial effect on the perception of flavours and aromas. For example, adding colour to an odorous solution can alter its olfactory characteristics such as its intensity and pleasantness, an effect that has now been reported in numerous studies (DuBose et al. 1980; Morrot et al. 2001; Zellner et al. 1991; Zellner and Kautz 1990). Interestingly, people are also far more likely to report that an odourless solution has an aroma if it is coloured rather than colourless (Engen 1972), suggesting that humans at least, have an intrinsic expectation that coloured solutions will possess an odour. Similarly, a study by Zellner and Kautz (1990) found that adding colour (e.g., red) to an odorous liquid (e.g., strawberry) enhances the perceived intensity of its odour. Zellner and Kautz also reported that some participants in their study simply refused to believe that coloured and uncoloured solutions of physically equal odour concentration were actually equally intense. Often, the perceived increase in the intensity of the odour depends on

the intensity of the colour that is added. For instance, Zellner and Whitten (1999) reported that people's rating of odour intensity of a mint-flavoured drink increased monotonically with increasingly higher intensity of green colouring. In a similar study, using artificial smells without any particular associations to foods or other objects, it was found that increasingly intense odours were associated with darker visual stimuli of the same colour (Kemp and Gilbert 1997).

In most previous experiments, colour-odour congruency has been assumed by virtue of a natural association (such as yellow - lemon) rather than formally tested using the methods of sensory evaluation. So far, one of the most thorough investigation of colour-smell correspondence has been conducted by Gilbert and colleagues (Gilbert et al. 1996) in a group of North American adults. In a series of experiments, the authors showed that participants reliably matched a variety of odours (essential oils and synthetic aroma compounds) to one or more specific colour names and that about a third of these matches remained the same after a two year interval. Subsequently, the same odours were used in a task where participants were required to match them to a colour using Munsell colour chips and it was found that thirteen of the twenty odours used were consistently matched to a specific hue (e.g., pine oil and green). In a similar study, Schifferstein and Tanudjaja (2004) recently showed that various perfumes are also non-randomly matched to colours, demonstrating that odour-colour congruency may exist even in the absence of any 'natural' associations.

The aim of this study was to investigate whether odours would be matched to their naturally corresponding colour (e.g., strawberry to red) and whether some of the previously reported colour-odour associations would also be present in a sample of UK university students. Given that systematic cross-cultural differences have been shown to exist for basic elements of odour perception, such as pleasantness and even

intensity judgments (Ayabe-Kanamura et al. 1998) and that colour-odour associations appear to be acquired rather than innate (Maga 1974), odour-colour correspondences might well vary across populations.

An additional aim of the study was to select suitable odour stimuli with robust colour associations that could subsequently be further investigated with fMRI.

6.2 METHODS

6.2.1 Participants

40 university students (19 females) took part in the study. The age range was 19 to 35 years with a mean age of 25 years. All of the participants had normal or corrected to normal visual acuity, intact colour vision, and no history of olfactory dysfunction.

6.2.2 Olfactory stimuli

Seventeen different odorants were used in the study (see Table 6.1). Five of the odorants (numbers 10 to 14) were chosen because they had previously been shown to elicit strong crossmodal odour-colour associations (Gilbert et al. 1996). Ten odours (numbers. 1 to 9 and 15) were essential oils of various fruits and herbs, extracted from natural sources. Their hypothesized colour association was based on the colour they naturally occur in (e.g., banana and yellow). Additionally one fragrance (Nr. 16) was chosen as a control, without any prior hypothesis about a colour association, because no object or food item was associated with it. Odorants 15 and 16 were supplied by Canvarson Ltd. (UK) and all other odorants were supplied by Quest International (UK).

Some of the odorants were diluted with diethyl phthalate (CAS number 84-66-2) which itself has minimal odour and dissolves a wide range of odorants (Gilbert et al. 1996) in order to match the odorants with respect to their intensity. 5ml of each odour solution was presented in an opaque brown Azlon® dispenser bottle (20ml). All of the bottles were individually labelled with a numeric code.

	<i>Odorant</i>	<i>CAS Number</i>	<i>Concentration</i>
1	Orange		100%
2	Strawberry		100%
3	Peppermint		50%
4	Grape		100%
5	Lemon		100%
6	Apple		100%
7	Banana		100%
8	Plum		100%
9	Spearmint		100%
10	Galbanum	8023-91-4	1%
11	Aldehyde C-16	77-83-8	100%
12	Cinnamic Aldehyde	104-55-2	80%
13	Methyl Anthranilate	134-20-3	50%
14	Caramel Furanone	28664-35-9	0.01%
15	Cucumber		100%
16	'Out at Sea'		100%
17	Benzaldehyde (Almond)	100-52-7	1%

Table 6.1: Odour stimuli.

The odorants used in the experiment together with their CAS number (where applicable) and their concentration in the non-odorous solvent diethyl phthalate.

6.2.3 Visual stimuli

The visual stimuli were produced by a PC-based programmable visual stimulus generator (VSG) card (Cambridge Research Systems Ltd.) and displayed on a 21" computer monitor. The computer screen was gamma corrected using Optical (Cambridge Research Systems Ltd.). The VSG system allows for well-defined colour presentation using the CIELAB colour space. This colour space specifies colour perception in terms of a three-dimensional space (L; a; b) with the L-dimension defining the lightness (from 0 =black to 100 = white) and the other two co-ordinates defining redness-greenness and yellowness-blueness, respectively.

Ten differently coloured rectangles (6cm wide x 8cm high) were displayed on the screen in two rows of five on a black background. The colours were luminance matched where possible to prevent participants from making their judgments based on brightness rather than hue. The yellow, orange and turquoise colours could not be displayed at the maximal luminance level achievable for blue and were displayed at a higher luminance instead. The colour co-ordinates in CIELAB colour space and the corresponding RGB values are shown in Table 6.2.

<i>Colour name</i>	<i>CIE co-ordinates (L; a; b)</i>	<i>RGB values</i>
Red	(10; 0.619; 0.346)	(231; 0; 0)
Yellow	(40; 0.413; 0.502)	(248; 248; 0)
Green	(10; 0.281; 0.601)	(0; 85; 0)
Blue	(10; 0.160; 0.127)	(0; 48; 255)
Orange	(21; 0.480; 0.410)	(255; 85; 12)
Pink	(10; 0.500; 0.100)	(255; 0; 193)
Brown	(10; 0.471; 0.458)	(98; 48; 0)
Turquoise	(30; 0.208; 0.316)	(0; 229; 189)
Purple	(10; 0.218; 0.120)	(99; 13; 253)
Grey	(10; 0.290; 0.300)	(56; 56; 56)

Table 6.2: Colour stimuli.

The 10 colours used in the study together with their corresponding CIE colour space co-ordinates and RGB values.

6.2.4 Procedure

The experiment took place in a large well-ventilated room equipped with an extractor fan above the testing site to facilitate the removal of the odours. The participants sat 50cm from of the computer monitor and were successively presented with each of the odour bottles. They were instructed to smell each odorant for as long

as they wanted and to select one of the 10 colours displayed on the screen that best matched the odour. The participants were required to mark each of their responses on a paper questionnaire. After the selection of a matching colour, they had to indicate the certainty of the match on a scale from 0% (very unsure) to 100% (absolutely sure). Participants then rated the smell itself on a 7-point rating scale with respect to pleasantness (very unpleasant – very pleasant), intensity (very weak – very strong) and familiarity (completely unknown – very familiar). They were then asked to write down the name of the odorant if they felt that they could identify it. Subsequently, the participants were asked to select a colour from the display which least matched the odour and to indicate the certainty of this judgment on the same scale as that used for the matching judgment. After these ratings had been collected, the next odorant was presented. All of the odorants were presented in a random order to each participant. All of the participants took a 5 minute break after each set of 6 odours.

6.3 RESULTS

As predicted, the majority of the odours (all except aldehyde C-16, methyl anthranilate, cucumber and 'out at sea') showed significant (non-parametric χ^2 test, $p < 0.01$) associations with one or more of the colours (see Table 6.3). The majority of these odours were matched to a colour they are naturally associated with (i.e., lemon-yellow). However, some of the odours were matched to colours that they were not naturally associated with (e.g., banana – pink). None of the odorants were matched above chance to the colours blue, purple or grey. On average, the participants were quite confident of their matches, as indicated by the mean reported certainty of 69% ($SD = 7.7$). A similar analysis of the least matching colour revealed that for all but 6 of the odourants (galbanum, aldehyde C-16, cinnamic aldehyde, methyl anthranilate, caramel furanone and benzaldehyde) significant associations of incongruence with one or more colours also existed (see Table 6.4). Most of the odours were matched to grey as the least fitting colour with the exception of peppermint and spearmint that were matched to red and the 'out of sea' odour that was matched to red and brown. The average certainty ($\bar{X} = 64.3$) for choices of colour mismatch was significantly lower (paired t-test, $t = 5.28$, $p < 0.001$) than for the colour match condition.

<i>Odorant</i>	<i>Hypothesized colour association</i>	<i>Observed colour association</i>	<i>Percentage of choices</i>	<i>Certainty (%)</i>
Orange	orange	yellow	50.0% **	87.5±10.7
Strawberry	red	orange	27.5% **	92.0±10.3
		pink	35.0% **	63.9±29.3
		red	27.5% **	72.7±23.3
		green	30.0% **	79.2±20.1
Peppermint	green	green	30.0% **	79.2±20.1
Grape	purple	pink	37.5% **	64.7±21.3
Lemon	yellow	yellow	77.5% **	87.7±13.6
Apple	green	green	42.5% **	75.9±21.5
Banana	yellow	pink	45.0% **	71.1±22.5
Plum	purple	pink	35.0% **	63.6±24.1
Spearmint	green	turquoise	40.0% **	73.1±25.2
		green	27.5% **	92.7±7.9
Galbanum	green	brown	35.0% **	66.4±13.9
		green	32.5% **	76.9±14.4
		purple	17.5% (n.s)	64.3±17.2
Aldehyde C-16	pink	brown	30.0% **	77.3±19.5
Cinnamic Aldehyde	red	orange	27.5% **	70.9±18.7
		pink	20.0% (n.s)	76.3±17.7
		purple	20.0% (n.s)	43.8±29.7
Caramel Furanone	brown	brown	72.5% **	73.5±19.0
Cucumber	green	pink	17.5% (n.s)	61.3±23.0
'Out at Sea'	no hypothesis	turquoise	22.5% (n.s)	67.8±19.2
Benzaldehyde	orange-	orange	32.5% **	66.9±26.9
	yellow			

Table 6.3: Predicted and observed colour-odour associations

The odorants used in the study are shown together with their hypothesized colour associations. The most frequently matched colours for each odorant are shown as well as the percentage of choices for that colour and the average certainty ($\pm$ SD) of the match measured on a scale from 0% (very unsure) to 100% (absolutely sure). In the case of a single distinct peak in the colour choice distribution, only one colour is shown. If two colours were chosen at a level significantly above chance, both colours are shown. If no colour was significantly often matched to an odour the most frequently matched colour is shown.

** = $p < 0.01$; (n.s.) = not significant

<i>Odorant</i>	<i>Colour mismatch</i>	<i>Percentage of choices</i>	<i>Certainty (%)</i>
Orange	grey	40.0% **	85.0±15.1
Strawberry	grey	35.0% **	75.0±20.7
Peppermint	red	32.5% **	69.2±21.0
Grape	grey	37.5% **	62.7±22.8
Lemon	grey	32.5% **	82.3±14.2
Apple	grey	30.0% **	74.2±15.6
Banana	grey	45.0% **	66.7±22.2
Plum	grey	37.5% **	65.7±20.6
Spearmint	red	35.0% **	71.4±17.5
Galbanum	red	20.0% (n.s.)	63.8±15.1
	turquoise	20.0% (n.s.)	60.0±26.2
Aldehyde C-16	pink	17.5% (n.s.)	60.0±21.6
Cinnamic Aldehyde	turquoise	20.0% (n.s.)	57.1±29.3
Methyl Anthranilate	red	20.0% (n.s.)	57.5±14.9
	grey	20.0% (n.s.)	65.0±18.3
Caramel Furanone	red	17.5% (n.s.)	61.4±24.8
Cucumber	grey	30.0% **	65.0±18.3
'Out at Sea'	red	35.0% **	71.4±18.3
	brown	25.0% **	67.0±20.6
Benzaldehyde	turquoise	20.0% (n.s.)	61.3±24.2

Table 6.4: Colour-odour mismatches.

The least matching colours for each odorant together with the percentage of choices for that colour and the associated certainty on a scale from 0% to 100% are shown. In the case of a single distinct peak in the colour choice distribution only one colour is shown. If two colours were chosen at a level significantly above chance, both colours are shown. If no colour was significantly often chosen as mismatching to an odour the most frequently selected colour is shown.

** = $p < 0.01$; (n.s.) = not significant

The odours used in the study were all perceived as at least moderately pleasant (Figure 6.1A) with an overall mean pleasantness rating of 4.56 (SD=1.58). A one-way analysis of variance (ANOVA) showed that there were significant differences between the pleasantness of the various odours ($F=9.61$, $p < 0.01$). However, a post-hoc Scheffé-test revealed that significant differences ($p < 0.05$, corrected for multiple

comparisons) existed only between the most pleasant odours lemon ($\bar{x} = 5.55, SD = 1.12$), orange ($\bar{x} = 5.55, SD = 1.06$), spearmint ($\bar{x} = 5.3, SD = 1.57$) and apple ($\bar{x} = 5.1, SD = 1.08$) and the most unpleasant odours caramel furanone ($\bar{x} = 3.33, SD = 1.59$), galbanum ($\bar{x} = 3.33, SD = 1.68$) and methyl anthranilate ($\bar{x} = 3.7, SD = 1.36$).

Overall, the odours were perceived as quite intense (see Figure 6.1B), with an average intensity rating of 4.81 ($SD=1.35$). ANOVA results confirmed that not all of the odours were perceived as being of equal intensity ($F=12.78, p<0.01$), although post-hoc tests again demonstrated that this effect was due to aldehyde C-16 ($\bar{x} = 2.88, SD = 1.42$) being perceived as significantly less intense ($p < 0.05$, corrected for multiple comparisons) than all other odours and methyl anthranilate ($\bar{x} = 3.68, SD = 1.40$), which was judged as being significantly weaker than the six most intense odours. No other differences were observed, demonstrating that all but two of the odours were perceived as being of equal intensity. This indicates that differences in colour-odour matches between the various odours are unlikely to be a result of differing odour intensities.

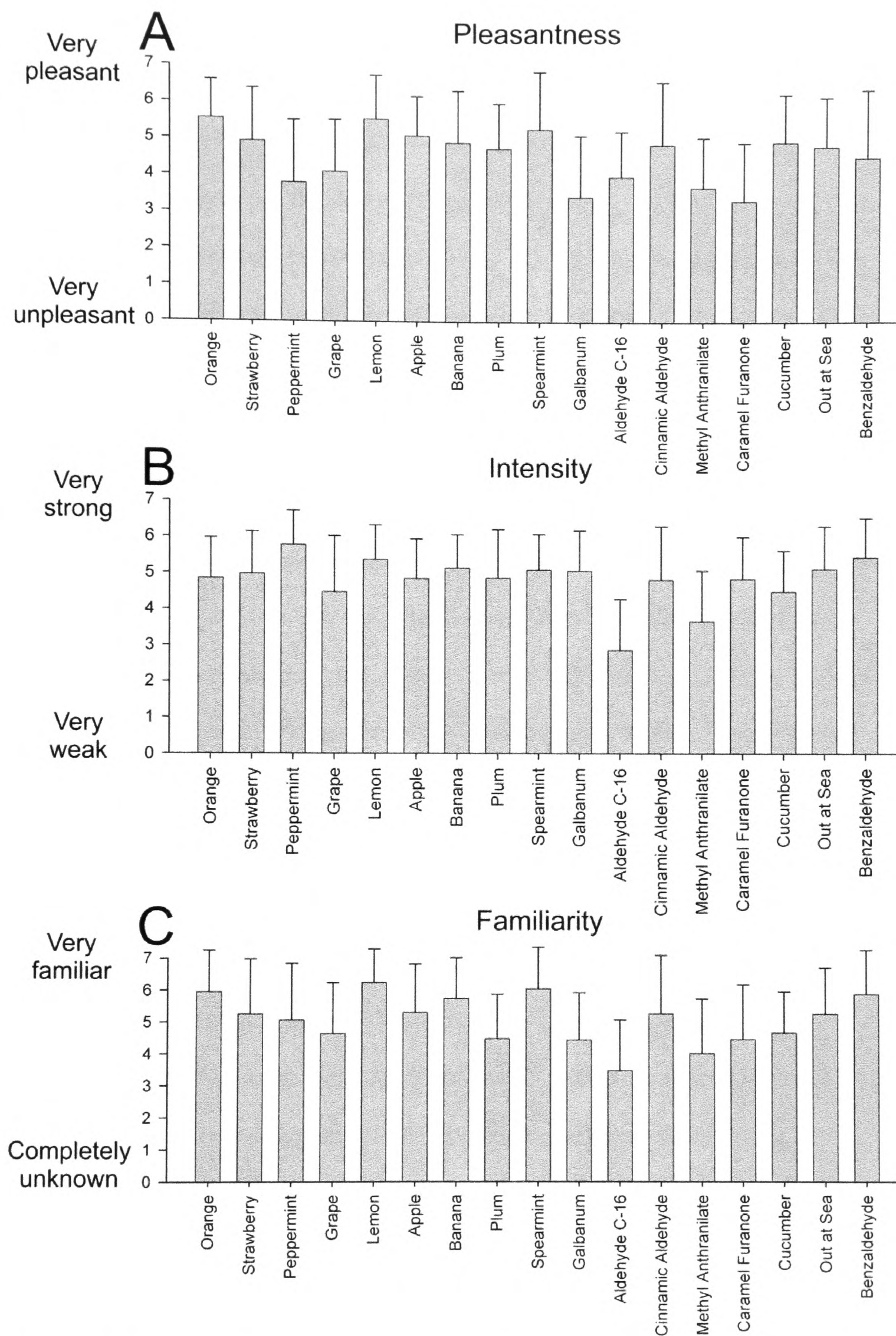


Figure 6.1: Odour pleasantness (A), intensity (B), and familiarity (C).

All ratings were on a 7-point scale and error bars show the standard deviation.
n = 40

Generally, the participants reported that they were familiar with most of the odours (see Figure 6.1C), as indicated by an average rating of familiarity of 5.06 ($SD=1.67$). However, not all odours were equally familiar to the participants as indicated by a significant ANOVA result ($F=10.37$, $p<0.01$). This was primarily due to the difference between the highly familiar odours of lemon ($\bar{x} = 6.23$, $SD = 1.05$), spearmint ($\bar{x} = 6.03$, $SD = 1.31$), and orange ($\bar{x} = 5.95$, $SD = 1.30$) and the most unfamiliar odours aldehyde C-16 ($\bar{x} = 3.46$, $SD = 1.59$) and methyl anthranilate ($\bar{x} = 4.0$, $SD = 1.71$), neither of which were identified by any of the participants. Interestingly, the participants were not very accurate at identifying the odours despite reporting subjectively that they were familiar with them. Only lemon, benzaldehyde (almond), spearmint and peppermint were correctly identified in the majority of cases (90%, 80%, 75%, and 73%, respectively). Notwithstanding the high familiarity with the banana smell ($\bar{x} = 5.73$, $SD = 1.28$) only two of the participants (5%) were able to identify it correctly.

Since there is evidence that people like odours more if they are familiar with them (Distel et al. 1999), we also tested for correlations between ratings of familiarity and pleasantness for each odorant (Table 6.5). Apart from two odours (Methyl anthranilate and 'Out at Sea') all odours showed a significant positive correlation between familiarity and pleasantness. Correlation coefficients ranged between $r = 0.33$ and $r = 0.75$ with strawberry and peppermint showing the strongest correlation ($r = 0.75$ and $r = 0.73$, respectively).

<i>Odorant</i>	<i>Pearson Correlation Coefficient</i>
Orange	0.56**
Strawberry	0.75**
Peppermint	0.73**
Grape	0.41**
Lemon	0.33*
Apple	0.61**
Banana	0.50**
Plum	0.56**
Spearmint	0.33*
Galbanum	0.45**
Aldehyde C-16	0.48**
Cinnamic Aldehyde	0.44**
Methyl Anthranilate	0.17 (n.s.)
Caramel Furanone	0.40*
Cucumber	0.50**
'Out at Sea'	0.25 (n.s.)
Benzaldehyde	0.58**

Table 6.5: Pleasantness × familiarity correlation.

The Pearson correlation coefficient of the ratings of pleasantness and familiarity are shown for each of the odorants.

** = $p < 0.01$; * = $p < 0.05$; (n.s.) = not significant

6.4 DISCUSSION

Most of the odours assessed in the present study showed significant colour associations. Of the 17 odours investigated in the experiment, only 4 did not show a significant colour association. For 8 of the odours, the colour associations were as predicted (strawberry = pink/red; peppermint = green; lemon = yellow; apple = green; spearmint = turquoise/green; galbanum = brown/green; caramel furanone = brown; and benzaldehyde = orange). The orange, grape, banana, plum and cinnamic aldehyde odours showed significant colour associations, although they were different from those that had been hypothesized prior to the study. Generally, 'sweet-fruity' smells had a tendency to be associated with the colour pink. The colours blue, purple and grey were never associated with any of the odours, possibly because their rare occurrence in foodstuffs (plums and grapes being an exception to this, however both of these aromas were associated with pink). All of the odours were perceived as above average with respect to their pleasantness and familiarity and both measures correlated for all but two of the odours. Interestingly, even though the participants in the study rated most odours as being quite familiar, identification accuracy remained rather poor. This phenomenon, known as the 'tip-of-the-nose' effect, where people are certain that they know the odour but fail to correctly name it has also been documented in several previous studies (Jonsson and Olsson 2003; Lawless and Engen 1977).

Both the failure to replicate previously shown colour-odour associations (Gilbert et al. 1996) for two of the odours (aldehyde C-16 and methyl anthranilate) and the observation of differing colour associations (cinnamic aldehyde was associated with brown-orange instead of the predicted red) may be due to cultural differences in the populations assessed in the two studies. It seems likely that the

American population studied by Gilbert et al. (1996), for example, would have associated the cinnamon smell with the red cinnamon-flavoured sweets that are common in North America, but not in the UK. By contrast, the sample of European participants tested in the present study appeared to associate the smell with the colours brown-orange, presumably because of the colour of cinnamon spice. Similar cross-cultural differences have previously been demonstrated for odour judgments of pleasantness and intensity (Ayabe-Kanamura et al. 1998) as well as for odour categorization responses (Chrea et al. 2005). Taken together, our findings also suggest that colour-odour associations may be acquired rather than innate.

The participants also found it more difficult to choose a non-matching colour for a given odour, which is reflected in the lower ratings of certainty in this condition and the fact that there are fewer significant colour mismatches than colour matches. This result provides the first empirical demonstration that certain colours can be perceived as particularly incongruent for a given odour. Generally, grey as the only 'non-colour' choice was only chosen as being non-matching to the odours. This might reflect a tendency to attribute colour to olfactory stimuli, a notion supported by findings that even inappropriately coloured odour solutions (i.e., strawberry-green) receive higher intensity ratings than colourless ones (Zellner et al. 1991; Zellner and Whitten 1999).

Many previous studies have investigated the influence of colours on flavour and taste perception (Alley and Alley 1998; Clydesdale 1993; King and Duineveld 1998; Kostyla and Clydesdale 1978; Maga 1974; Zellner and Durlach 2003), but the relative importance of olfaction to these effects remains relatively unclear. However, the relative contribution of olfaction to perceived flavour is quite substantial: It has been shown experimentally that up to 80% of the overall reported 'taste' of a solution

disappears if the nose is blocked (Murphy et al. 1977). Even though it has been shown that perceived sweetness can be modulated by colour cues (Maga 1974) these effects appear to be less robust (Alley and Alley 1998) than those of colour-induced odour enhancement. Taken together, these results suggest that it may be the olfactory component of flavour perception in particular, that is primarily modulated by visual cues.

Most previous studies that have investigated odour-colour interactions presented stimuli in the form of coloured solutions containing a dissolved odorous substance (DuBose et al. 1980; Morrot et al. 2001; Zellner et al. 1991). Even though this mode of stimulus delivery possesses the undoubted advantage of facilitating multisensory binding processes - since both visual and olfactory stimuli are presented simultaneously and originate from the same spatial location, this method can not easily be adopted for use in studies investigating timed responses to any of the stimulus characteristics.

Additionally, it could be argued that at least some of the effects observed in studies using coloured liquids may be attributed to the mere co-localization of olfactory and visual stimuli, for it is known that this facilitates multisensory integration (Calvert et al. 2004; Wallace et al. 2004b). Previous studies suggest that colour appropriateness has a relatively small influence on colour-induced odour enhancement and that the presence of colour per se can produce these effects (Engen 1972; Zellner and Whitten 1999). However, it may well be that effects of colour appropriateness exist but are small in comparison to co-localization effects. Therefore, it would be interesting in future experiments to investigate whether colour-induced odour enhancement still occurs if both odour and colour are presented simultaneously but from spatially disparate locations.

Furthermore, it is also well known that multisensory enhancement effects tend to be more pronounced if stimuli are presented at threshold level (Dalton et al. 2000; Stein 1998), rather than for clearly suprathreshold stimuli, as used in the present study. Therefore one would expect to find a stronger effect of colour-appropriateness for weak odours. I speculate that colour-odour congruency -rather than spatial co-localization, would contribute more substantially to these effects under such experimental conditions.

Based on the findings of this study, I selected 4 odorants for further investigation using fMRI (see Chapter 7). The selection criteria used were that the odours needed to show a significant colour association in a sample of UK students and that the associated colours were of a different hue for each odour. As a result, the odour-colour pairs strawberry-red, spearmint-turquoise, caramel furanone – brown and lemon –yellow were chosen for the fMRI study presented in the next chapter.

7 Multisensory colour-odour integration

Colour has a profound effect on the perception of odours. For example, strawberry flavoured drinks smell more pleasant when coloured red than green and descriptions of the “nose” of a wine are dramatically influenced by its colour. Using functional magnetic resonance imaging (fMRI) I demonstrate a neurophysiological correlate of these crossmodal visual influences on olfactory perception. Participants were scanned while exposed either to odours or colours in isolation, or to colour-odour combinations that were rated on the basis of how well they were perceived to match. Activity in caudal regions of the orbitofrontal cortex and in the insula cortex increased progressively with the perceived congruency of the odour-colour pairs. These findings demonstrate the neuronal correlates of olfactory response modulation by colour cues in brain areas previously identified as encoding the hedonic value of smells.

7.1 INTRODUCTION

In everyday life odours are often perceived together with visual cues. Both types of sensations interact to modulate the subjective experience of the stimuli from which they emanate. Numerous behavioral studies demonstrated that the ability to correctly identify an odour relies heavily on visual inputs. For example, if subjects are presented with olfactory cues alone, only a few of the presented odours are identified

correctly (Desor and Beauchamp 1974). There is a general lack of awareness of how difficult it can be to identify a substance by its odour alone because visual cues are usually present to facilitate identification. One visual feature with a particularly strong influence on odour perception is colour. The colour of a fruit, for example, provides an important visual cue about its ripeness and palatability.

Several reports of strong associations between certain odours and colours (Gilbert et al. 1996; Zellner and Kautz 1990) imply that such colour-smell associations are most likely acquired and may be subject to variation between cultures. Nonetheless, some of these associations are particularly robust across subjects (e.g. yellow - lemon). Several colour-smell associations are so compelling that an odour percept can change with colour, e.g. subjects may perceive a cherry-flavoured drink as orange-flavoured if it is coloured orange (DuBose et al. 1980). Furthermore, colour not only facilitates odour identification, but can also influence judgments of odour intensity and pleasantness. For example, both the perceived intensity and pleasantness of an appropriately coloured solution (e.g. red-strawberry) is judged as higher than that of an inappropriately coloured (e.g. green-strawberry) or a colourless solution, even when the actual concentration of odorant remains constant (Zellner et al. 1991). More recently it has been found that tasteless red colouring added to a white wine induces a perceptual olfactory illusion that causes the wine to be described with olfactory terms typically used only for red wines (Morrot et al. 2001).

Despite the plethora of behavioral studies showing the influence of colour on various olfactory judgments, the neurophysiological basis for these crossmodal interactions is still very poorly understood. In mammals, projections from the retina and olfactory bulb have been found to converge in the piriform cortex, the olfactory

tubercle, the cortical region of the medial amygdala and the lateral hypothalamus (Cooper et al. 1994). In primates, a prominent region for multisensory integration of olfactory and visual signals is the orbitofrontal cortex (OFC), where afferent inputs from both primary olfactory cortex and higher order visual areas converge (Ongur and Price 2000). Additionally, populations of bimodal neurones responsive to both visual and olfactory stimulation have been reported within the OFC of non-human primates (Rolls and Baylis 1994). Recently, a study using functional magnetic resonance imaging (fMRI) in humans reported that congruent picture-odour combinations (such as the image of a bus and the smell of diesel) enhance neural activity, in the OFC and hippocampus (Gottfried and Dolan 2003). Whether similar regions participate in the synthesis of odours and lower level visual features such as colour, remains to be explored.

Although the precise neuronal mechanisms underlying the integration of visual and olfactory cues have not yet been studied in detail, some general principles characterising crossmodal integration at the neuronal level have emerged from the study of other combinations of sensory modalities (Stein 1998). Specifically, when two or more sensory cues occur at the same time and in approximate spatial correspondence, the firing rate of a multisensory neuron to a stimulus in one modality can be measurably altered by the presence of a second stimulus in another modality. This is referred to as “multisensory integration”. Rarely, this crossmodal modulation of the cell’s output can exceed the sum of its response to either modality in isolation - a phenomenon referred to as superadditivity. Such superadditive responses are not observed under all bimodal stimulus conditions but are also influenced by factors other than temporal and spatial concurrence. The most important factor influencing superadditivity is the intensity of the stimuli, since the greatest effects of non-linear

enhancements in neural firing rates and the greatest behavioural gains are found for multisensory combinations of the ‘weakest’ sensory stimuli (Meredith and Stein 1986). Based on this phenomenon -termed the principle of inverse effectiveness, many electrophysiological studies have presented their stimuli near threshold level. Superadditive multisensory interactions have also been identified in the context of human neuroimaging experiments involving the chemical senses (de Araujo et al. 2003c; Gottfried and Dolan 2003).

In addition to the detection of crossmodal superadditive responses, systematic manipulation of the perceived congruency of two multisensory cues have also been shown to affect the height of the blood oxygen level dependent (BOLD) response. In particular, increasingly better matched crossmodal cues induce a corresponding enhancement of the haemodynamic response in areas thought to be involved in their combination. Parametric imaging designs such as these avoid some of the interpretation issues associated with superadditive interactions (see Calvert, 2000 and below).

This study used fMRI to investigate whether similar multisensory mechanisms might underlie the interaction of visual colour and olfactory cues in the generation of an olfactory percept in humans. Based on findings of previous human neuroimaging studies of olfaction by others (Anderson et al. 2003; Gottfried et al. 2002a; Gottfried and Dolan 2003; Rolls et al. 2003a; Zald and Pardo 2000; Zatorre et al. 1992), I hypothesised that if olfactory responsive areas including the piriform cortex, amygdala and OFC participate in the integration of odour-colour cues, activity in these regions should increase systematically with increasingly better matched odour-colour pair trials, as rated on an individual and subjective basis.

7.2 METHODS

7.2.1 Subjects

Ten healthy right-handed volunteers participated in this study (6 females and 4 males; mean age 27 years, age range 22-35 years). All participants gave written informed consent after having received the instructions for the study. One subject had to be discarded from the study because of excessive motion during scanning. Data analysis at the group level thus includes nine subjects. The study was approved by the Central Oxford Research Ethics Committee (C99.179).

7.2.2 Stimuli and task

The four colour-odour pairs showing the most consistent match across subjects in the behavioural experiment (Chapter 6) and being associated to different colours were used in the fMRI study. These congruent pairings were yellow-lemon, strawberry-red, spearmint-turquoise and caramel-brown and were presented during scanning as well as the variably less congruent pairings of each odour with the remaining three colours. The four smells were highly matched for pleasantness, intensity and familiarity (Table 7.1).

	<i>Lemon</i>	<i>Strawberry</i>	<i>Spearmint</i>	<i>Caramel</i>
Pleasantness	5.6±1.2	4.9±1.5	5.3±1.6	3.3±1.6*
Intensity	5.4±1.0	5.0±1.2	5.1±1.0	4.9±1.2
Familiarity	6.2±1.1	5.3±1.7†	6.0±1.3	4.5±1.7‡

Table 7.1: Ratings of pleasantness, intensity and familiarity for the 4 odours.

Values are averages ± SD of the behavioural ratings on a 7-point scale for 40 subjects.

* significantly ($p<0.05$) different from the remaining three odours.

† significantly ($p<0.05$) different from lemon.

‡ significantly ($p<0.05$) different from lemon and spearmint.

During scanning, subjects were presented for 6 s with either

1. a visual stimulus (yellow, red, turquoise or brown),
2. an olfactory stimulus (lemon, strawberry, spearmint or caramel) or
3. a bimodal visual-olfactory stimulus.

The order of each of the three conditions was randomised. Each presentation was followed by a rest period of 30s to avoid habituation to the odour stimuli. For the unimodal conditions, each of the 4 olfactory and colour stimuli was presented 3 times. For the bimodal conditions, each odour was presented 3 times with a congruent colour (i.e. lemon-yellow) and once with each of the remaining 3 colours. Overall, 12 visual, 12 olfactory and 24 bimodal stimuli were presented over a period of 28 min 48s.

Before scanning, subjects were informed that odours might occur in isolation or in the presence of a simultaneously presented colour. In addition, colour patches could also occur in the absence of an odour. They were also told that the order of these events would be random so they should maintain their attention to both channels equally throughout the experiment. Subjects were also instructed that on the bimodal

conditions only, they were to rate each colour-smell combination with respect to 'goodness of fit between colour and smell' on a rating scale ranging from 1='very good' to 4='very bad' using a 4-button response box. Rating responses were cued by the word RATE presented 15s after the end of each bimodal presentation. To control for the motor response to the visual cue between the bimodal and unimodal conditions, the same visual cue also appeared 15s after each unimodal presentation and subjects were instructed to press any of the 4 buttons. The advantage of a parametric design that requires a crossmodal congruency judgment is, that it allows multisensory colour-odour integration sites to be identified in the covariance analysis between bimodal trials and rating judgments. However, as the same judgment cannot be made in the unimodal conditions (for example, it is not possible for humans to rate the congruency between two simultaneously presented odours), contrasts between the bimodal and unimodal conditions may be confounded by differences in the task requirements. Therefore superadditive effects $[OV - (O + V)]$ must be interpreted cautiously.

Full screen colour images were generated using a video projector located outside the scanner room and projected onto a translucent screen placed directly outside the magnet bore. Subjects wore prism glasses so that they could see the screen while lying in the scanner. Olfactory stimuli were delivered using a custom-built, computer-controlled olfactometer as described in Chapter 2. This was identical in design to that used by Rolls and colleagues (for a detailed description see Rolls et al. 2003) so that switching between odorised and odourless air was free of any auditory, tactile or thermal cues that could have alerted the subjects to the onset of odour delivery. Subjects were asked to breathe normally through their nose for the duration of the scan while an air-stream of either odorised or odourless air was delivered at a

flow rate of 6 l/s through a Teflon tube placed directly under the subject's nose. They were also informed that although during most presentations, the visual stimulus would be accompanied by an odour, they should refrain from sniffing at the onset of a colour cue and continue to breathe normally. This instruction was included to avoid sniff related activation of olfactory areas (Anderson et al. 2003) in the colour-alone condition. Furthermore, in the absence of a colour cue in the odour-alone condition, sniffing only at the onset of trials containing a colour stimulus could have resulted in a potential confound of the comparison between bimodal colour-odour and odour-alone conditions. Instead, during the scan, subjects were asked to detect the onset and offset of odours and to signal these events by making an appropriate ON/OFF button response. This method resulted in a slight jitter in the onset and duration of the odour trials that was taken into account for data analysis.

7.2.3 Data acquisition and analysis

Both functional and structural MRI images were acquired on a Siemens/Varian 3T system fitted with a birdcage head coil. For the functional data series, a total of 581 T2* weighted echo-planar imaging (EPI) volumes were taken over a time period of 28min 48s. Each volume consisted of 25 continuous oblique coronal slices that were tilted $\sim 20^\circ$ towards the axial plane with an in-plane resolution of 3x2.5mm and a thickness of 3mm, covering the anterior half of the brain dorsally reaching a y-coordinate of +4 of the Montreal Neurological Institute (MNI) standard space and a ventral coordinate of $y = -12$. This allowed coverage of all primary and secondary olfactory areas in OFC and the temporal lobes. Other imaging parameters were: TR=3s, 64x64 matrix, FOV 192x160 mm, TE=25ms, flip angle 90° . In order to

minimize susceptibility artifacts in orbitofrontal brain regions, slices were acquired in the oblique coronal orientation (O'Doherty et al. 2003) with a short echo time of 25ms and a small in-plane voxel size. Additionally an automated frontal weighted local shimming method was used (Wilson et al. 2002a). This imaging protocol is optimized for fMRI studies of OFC function and has been extensively used by other groups on the same scanner (Rolls et al. 2003a).

For registration into standard space, a whole brain T2* weighted EPI volume (54 slices, TR=7s) and a high-resolution, whole brain T1 weighted morphological scan (1.5mm slice thickness, 1.5mmx1.5mm in-plane resolution) was acquired after the experimental paradigm.

Statistical image analysis was carried out using the FMRIB Software Library (www.fmrib.ox.ac.uk/fsl). The initial strategy for analysing the bimodal condition was to first test for a linear correlation between the blood oxygen level dependent (BOLD) response and ratings of the congruency between smells and colours, and then to assess superadditivity (i.e. where the activation to bimodal stimuli exceeds the sum of both unimodal stimuli). Prior to data analysis, the first 3 volumes were deleted. Analysis was carried out using FEAT, the FMRIB Expert Analysis Tool. The following pre-processing was applied: motion correction using MCFLIRT (Jenkinson et al. 2002); spatial smoothing using a Gaussian kernel of FWHM 5mm; mean-based intensity normalization of all volumes by the same factor; non-linear highpass temporal filtering (Gaussian-weighted LSF straight line fitting, sigma=36.0s). Each of the 3 event types (visual, olfactory and bimodal) was modelled as a separate explanatory variable. A fourth explanatory variable with the same time course as the bimodal one was used to model the linear correlation between the BOLD response and the behavioral rating for the degree of matching. This variable was orthogonalised with

respect to the bimodal one. Statistical analysis was carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich et al. 2001).

For group analysis, the individual results were registered both to high-resolution anatomical MR images and to the MNI 152 standard image. Registration to high resolution and standard images was carried out using FMRIB's Linear Image Registration Tool (FLIRT) (Jenkinson et al. 2002). Mixed-effects (often referred to as 'random-effects') group analysis was carried out using FMRIB's Local Analysis of Mixed Effects (FLAME) software (Beckmann et al. 2003) with a cluster threshold of $Z > 2.0$ and a cluster significance threshold of $P = 0.05$ (corrected for multiple comparisons) (Forman et al. 1995; Friston et al. 1994; Worsley et al. 1992). This threshold was specifically chosen on the basis of pilot experiments that showed robust activation in olfactory areas in both temporal and orbitofrontal cortices using these parameters.

7.3 RESULTS

7.3.1 Behavioural

Analysis of the behavioural responses obtained during the fMRI experiment showed that for 3 odours (lemon, spearmint and caramel) the colour-smell combinations rated as most congruent in our preliminary experiment were indeed rated as significantly (Student t-test $p<0.05$) better matching than combinations of the same odour with any of the other three colours (Table 7.2). Although the strawberry odour showed a trend towards higher perceived congruence with red than the other 3 colours, the only statistically significant difference occurred between red and the turquoise. Note however, that for the FMRI data analysis, each individual congruency rating for a bimodal trial was used to model the BOLD response (i.e. if a subject perceived the combination spearmint-brown as a very good match, then this was accounted for in the analysis model).

	<i>Lemon</i>	<i>Strawberry</i>	<i>Spearmint</i>	<i>Caramel</i>
Yellow	1.1±0.3*	2.6±1.0	3.8±0.4	3.2±0.8
Red	3.2±0.7	2.0±1.0†	3.2±1.1	4.0±0.0
Turquoise	3.1±0.6	3.0±0.9	1.6±1.1*	3.1±1.1
Brown	2.4±1.1	2.3±0.7	2.3±0.5	1.9±0.9*

Table 7.2: Ratings of congruence for colour-odour pairings.

Values are averages $\pm$ SD of the behavioural ratings of all colour-odour combinations presented during the experiment on a 4-point scale (1='very good' match and 4='very bad' match) for all 9 subjects.

* significantly ($p<0.05$) different from the remaining three colours.

† significantly ($p<0.05$) different from turquoise.

7.3.2 Unimodal stimulation

Presentation of odours alone produced bilateral activation in the piriform cortex/amygdaloid region ($x, y, z = 14, -6, -30$; Z-score = 3.81 and $x, y, z = -22, -2, -18$; Z-score = 3.29) and the putamen ($x, y, z = 14, -4, -10$; Z-score = 3.83 and $x, y, z = -6, 2, -8$; Z-score = 4.22) (Figure 7.1A). Lateralised activation was observed in the right orbitofrontal gyrus ($x, y, z = 24/32/-24$; Z-score = 4.38) and in the left insula cortex ($x, y, z = -44, 2, -14$; Z-score = 3.49). Both piriform and orbitofrontal areas are considered to be primary and secondary olfactory cortices in primates (Tanabe et al. 1975) and have been shown to activate in response to odorants in human neuroimaging studies (Zald and Pardo 2000). There was no significant activation of these regions when colours were presented alone.

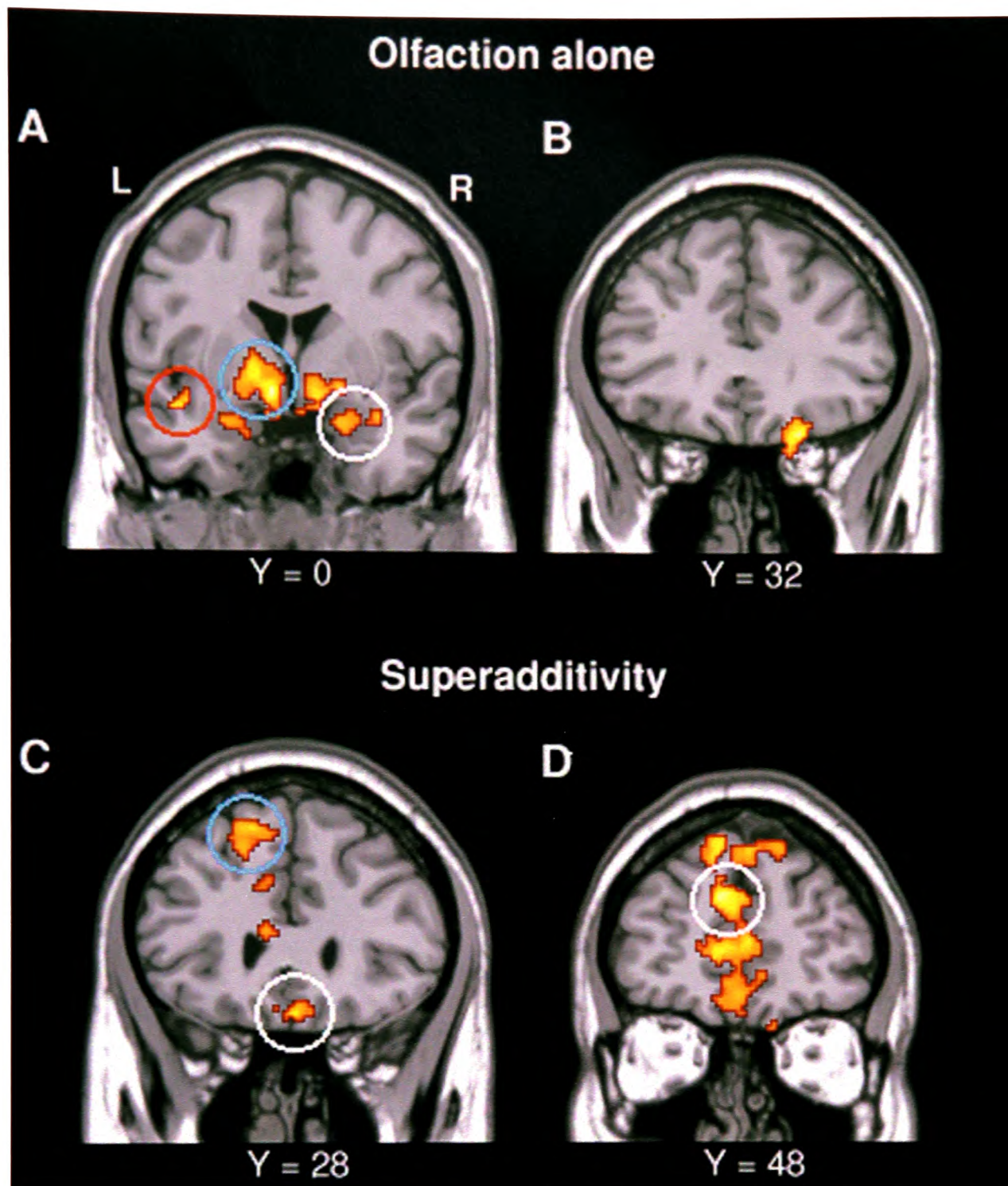


Figure 7.1: Odour activation and superadditivity.

Group response to odour stimulation alone and brain regions displaying effects of superadditivity [bimodal - (vision + smell)]. Olfactory stimulation (A) evoked neural responses bilaterally in the piriform cortex/amygdala (white), the ventral striatum (blue) and the left insula cortex (red) as well as in (B) the right orbitofrontal cortex. Superadditive responses were observed in (C) medial orbitofrontal cortex (white) and the superior frontal gyrus (blue) and (D) the anterior cingulate (white).

Slices y-coordinates are in the MNI standard space and the right side of the brain is shown on the right side of the pictures.

7.3.3 Superadditivity

To assess whether indices of multisensory interactions similar to those previously reported in electrophysiological (Stein 1998) and neuroimaging experiments (Calvert 2001) could be identified in the current imaging experiment, I compared the BOLD response to odour-colour pairings to the activation of odours and colours alone [bimodal - (odours + colours)]. This included the behavioural ratings in the overall model, so that the height of the BOLD response to a ‘very good fit’ was modelled as larger than the response to a ‘very bad fit’. Several regions displayed effects of superadditivity (Figure 7.1 C and D): the medial wall of the superior frontal gyrus ($x, y, z = -8, 48, 26$; Z-score = 4.48), the superior transverse frontopolar gyrus extending caudally into the anterior cingulate cortex ($x, y, z = 4, 48, 8$; Z-score = 3.96 and $x, y, z = -10, 48, 4$; Z-score = 3.83) and the OFC along the gyrus rectus ($x, y, z = 4, 28, -19$; Z-score = 3.16 and $x, y, z = -4, 35, -18$; Z-score = 3.07).

7.3.4 Bimodal modulation and congruency

Using the ratings of relative colour-odour congruency as a parametrically varying explanatory variable for brain activation changes, a network of brain areas exhibiting increasing activity with progressively higher perceived congruency was identified (Figure 7.2). This network was entirely left lateralised and was mainly localized within the caudal orbitofrontal cortex around the olfactory sulcus ($x, y, z = -16, 32, -4$; Z-score = 3.88), inferior frontal gyrus pars orbitalis ($x, y, z = -20, 22, -16$; Z-score = 2.92) and gyrus rectus ($x, y, z = -8, 16, -24$; Z-score = 3.34). Other brain regions exhibiting the same colour-odour modulations were found in the anterior

insula cortex ($x, y, z = -32, 22, -8$; $Z\text{-score} = 2.75$), the frontal operculum ($x, y, z = -54, 24, 4$; $Z\text{-score} = 2.68$) and the temporal pole ($x, y, z = -56, 18, -8$; $Z\text{-score} = 3.03$).

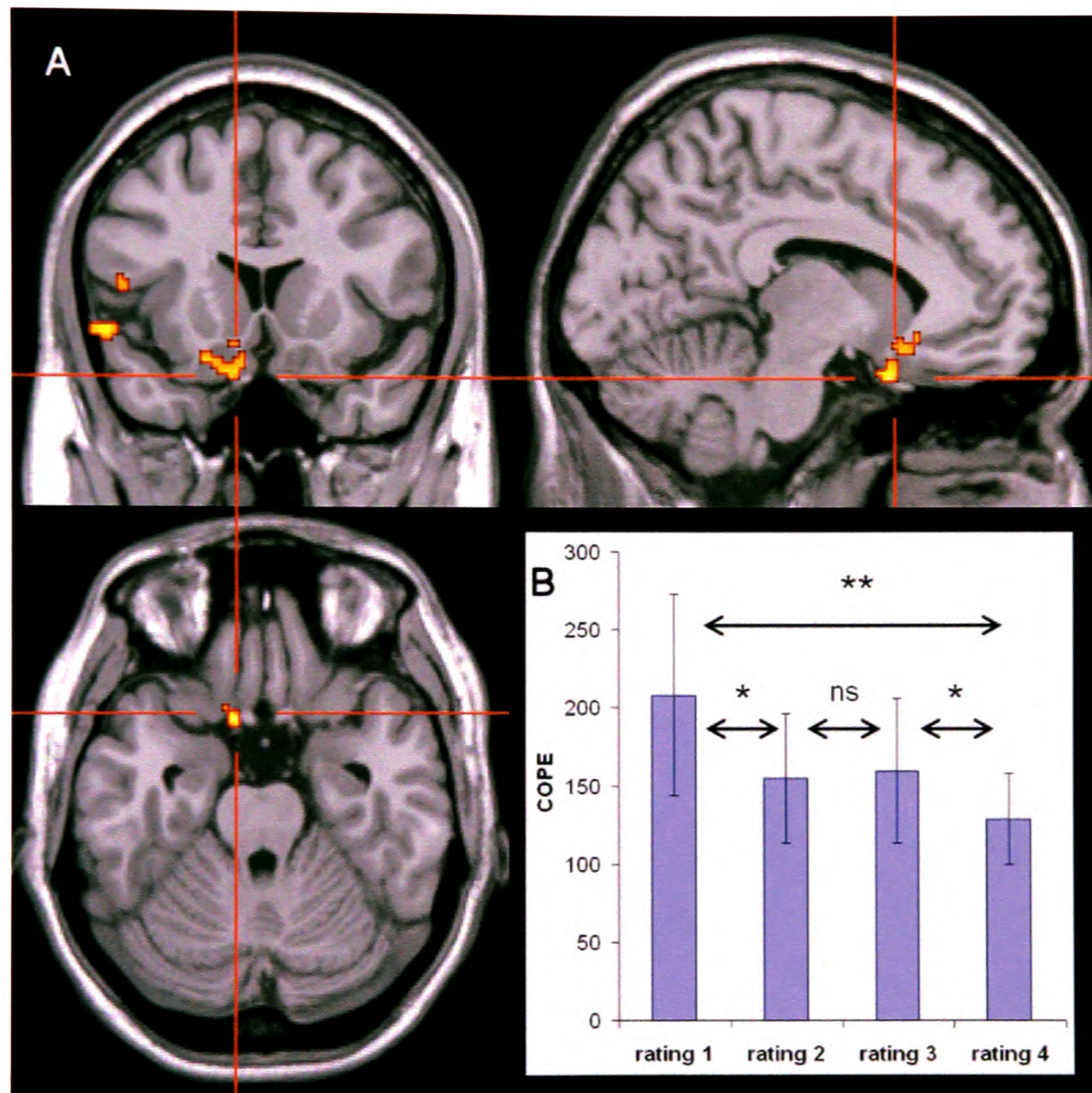


Figure 7.2: Brain regions showing odour-colour modulation.

A The group activation map shows the main cluster of activation (red crosshairs) in the caudal region of the left OFC ($x, y, z = -8, 16, -24$; $Z\text{-score} = 3.34$) in all three slice orientations. Here, the BOLD signal increased with greater perceived odour-colour congruency. All activated voxels were rendered onto the standard Montreal Neurological Institute (MNI) brain and the right side of the brain is shown on the right side of the pictures.

B The panel shows the parameter estimates for smell alone and the 4 different degrees of congruency of the colour-odour pairs (ranging from 1 = 'very good' to 4 = 'very bad') averaged across all 9 subjects. Black lines indicate the standard error of the mean and asterisks significance levels of t-tests (**= $p < 0.01$, *= $p < 0.05$ and ns=not significant).

7.3.5 Neural suppression and incongruency

Incongruency in stimulus properties has been shown to suppress neuronal activity for audio-visual stimulus combinations (Calvert 2001) and congruent olfactory-taste stimuli have been reported to cause neural suppression (Small et al. 1997). To investigate whether similar effects are observable for olfactory-visual stimuli, we tested the linear correlation of the BOLD response with incongruency (i.e. brain areas that respond increasingly stronger the more incongruent the stimulus combinations are) and did not find an effect. Similarly, incongruent visual-olfactory stimuli did not significantly decrease the BOLD response below the levels observed to olfactory stimuli alone (Figure 7.3).

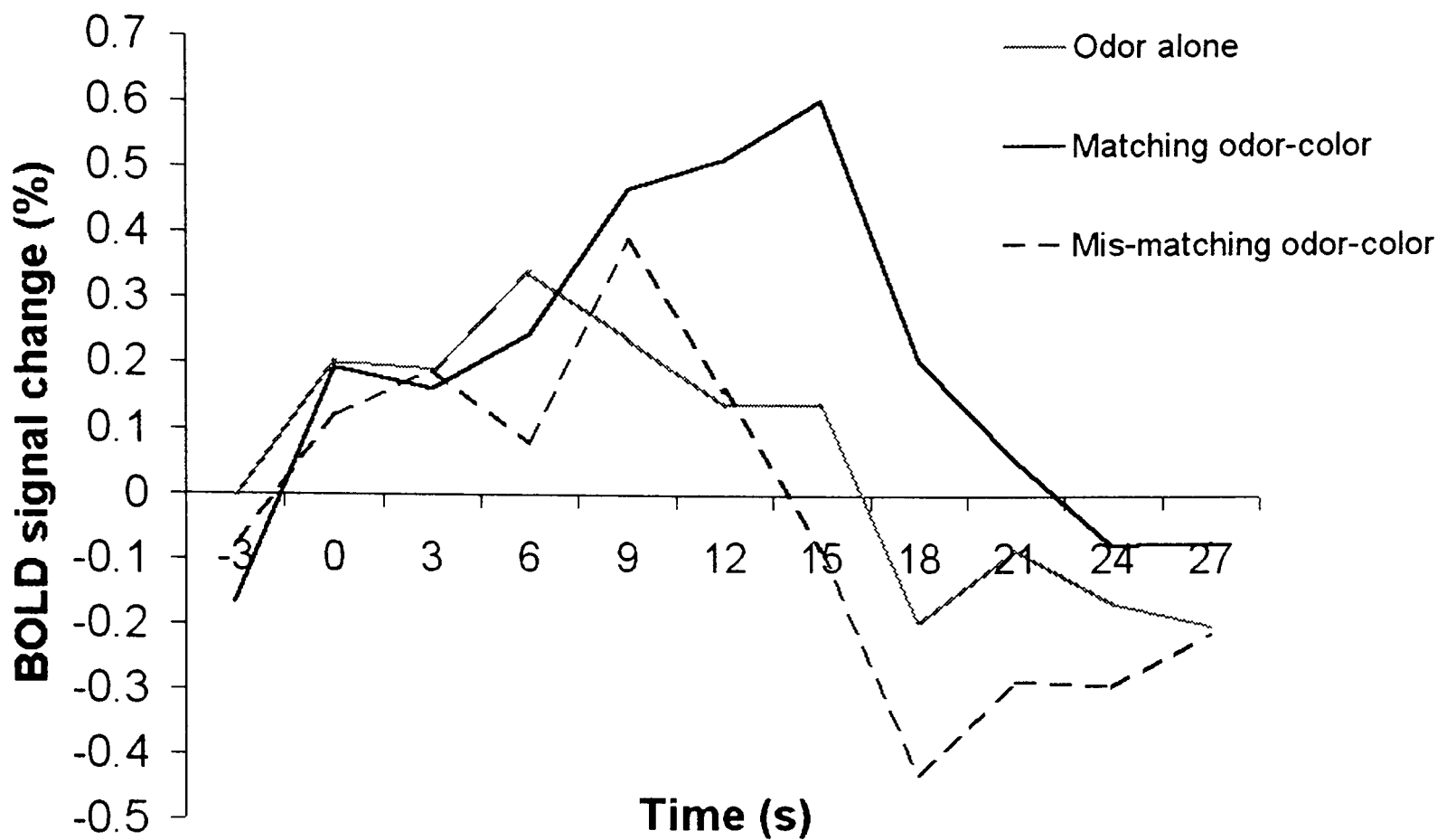


Figure 7.3: Time course of activation.

The chart shows the group ($n=9$) averaged time course of the percentage BOLD signal changes for both matching (rating of 1) and mismatching (rating of 4) odour-colour trials as well as the odour alone trials in the peak voxel within the left OFC ($x, y, z = -8, 16, -24$). Stimulation started at time 0 and lasted for 6 s.

As can be seen, congruent odour-colour pairs elicited a significantly greater BOLD response than that observed to either incongruent pairs ($t= 3.05$, $df=8$, $p<0.01$) or odours in isolation ($t= 3.26$, $df=8$, $p<0.01$). Note, differences in the time to peak across conditions are most likely to reflect corresponding differences in BOLD amplitude (Miezin et al. 2000).

7.4 DISCUSSION

The aim of this study was to probe the neurophysiological basis of visual influences on odour perception using fMRI. The presentation of odours in the absence of visual cues was first shown to stimulate the piriform/amygdaloid region, the right OFC and left insula cortex – consistent with previous olfactory neuroimaging experiments (Anderson et al. 2003; Gottfried et al. 2002a; Poellinger et al. 2001; Rolls et al. 2003a; Zald and Pardo 2000). These areas are understood to correspond to human primary olfactory and associative olfactory cortices respectively. A subset of these olfactory-responsive areas was also found to be sensitive to the perceived congruency of bimodal odour-colour trials. Specifically, activation in the orbitofrontal and insula cortices increased in strength with increasingly higher ratings of the perceived congruency between specific odours and colour patches. These findings are consistent with the hypothesis that colour modifies the perception of odours at a relatively late stage of olfactory processing in heteromodal regions of the OFC and a region of the insula cortex previously implicated in flavour processing (de Araujo et al. 2003c).

The detection of crossmodal modulatory effects in a network of areas including the OFC and insula cortex suggests that colour may influence different aspects of olfactory processing. The OFC receives converging projections from multiple sensory modalities, including the primary olfactory cortex and the ventral visual pathway (Ongur and Price 2000; Rolls and Baylis 1994), making it a plausible initial site of information exchange between the visual and olfactory systems. Electrophysiological studies in non-human primates and imaging experiments in humans have implicated the OFC in stimulus-reinforcement associative learning using olfactory and visual stimuli (Gottfried et al. 2002c; 2003). For example, responses in

the OFC to olfactory, gustatory or visual cues previously associated with certain foods, are diminished when subjects are fed to satiety (Rolls 2001) and activity in this region has been shown to increase according to the perceived pleasantness of olfactory stimuli (Anderson et al, 2003). Together, these data contribute to the mounting evidence that it is specifically the rewarding properties of the sensory cues that are represented in the OFC. The systematic BOLD increase observed in the current study is therefore likely to reflect the physiological mechanism underlying the behavioural phenomenon whereby the more appropriate the colour-odour combination, the greater is the perceived pleasantness of the odour (Zellner et al, 1991).

In addition to changing the hedonic value of odours, colours have also been shown to influence the perception of flavour (Rolls et al. 1982). Visuo-olfactory modulated responses in the current experiment were also detected in the left anterior insula cortex and the adjacent frontal operculum. These regions have previously been identified as areas of primary taste cortex in monkeys (Plata-Salaman et al. 1995; Yaxley et al. 1990) and are consistently activated during human imaging studies of taste (Frey and Petrides 1999; O'Doherty et al. 2001; Small et al. 2003; Small et al. 1999b). However, the agranular insula, located at the caudal border of the OFC, is also responsive to olfactory inputs (Weismann et al. 2001) and neuroimaging studies of olfaction have reported activation in this region (Cerf-Ducastel and Murphy 2001; de Araujo et al. 2003c; Poellinger et al. 2001; Savic et al. 2002). Interestingly, this anterior region of the insula appears to be multimodal, i.e. it responds to both retronasal and orthonasal olfactory stimuli as well as trigeminal inputs and pure tastes, leading to speculation that it must be involved in the cortical representation of flavour (de Araujo et al. 2003c; Savic et al. 2002). Additionally, the insula receives inputs

from the ventral visual stream and has been shown to be involved in the multisensory integration between vision and touch (Hadjikhani and Roland 1998; Prather et al. 2004). However, to the extent that our experience of flavour is predominantly derived from our sense of smell, it seems highly plausible that colour cues do not only modulate odour representations, but also the putative taste/flavour sensations that may be automatically retrieved in the presence of food odours. In the current study then, I believe crossmodal modulations have an effect not only in areas of visual-olfactory processing, but also drive the observed enhancement of neuronal activity in adjacent downstream regions involved in flavour perception such as the insula cortex.

A topical issue of debate in the multisensory literature relates to the appropriateness of different analytic criteria used for identifying a putative multisensory integration site. One relatively well-established approach has been to expose subjects to bimodal cues, matched or mismatched along some parameter (e.g. time, space or content) as well as to each modality independently, and look for brain areas exhibiting positive and negative statistical interactions to congruent and incongruent bimodal cues respectively [$OV > O+V$] and [$OV < \text{either } O \text{ or } V$, whichever is the greater]. This strategy represents a reasonable attempt to capture BOLD responses that bear some resemblance to the known response properties (crossmodal response facilitation and suppression) of multisensory neurones. However, there are clearly theoretical complications involved in translating the behaviour of individual neurones to neuronal population responses detected by fMRI (see Calvert, 2001 for a review). In the current study, I adopted an alternative strategy for identifying multisensory integration sites, which was to present bimodal odour-colour cues that were perceived to match to a varying degree, and look for brain areas showing response changes that were correlated with the extent of perceived

congruency. Parametric designs such as these avoid some of the inherent problems associated with calculation of interactions designed to mirror crossmodal response facilitation and suppression at the cellular level. One such problem relates to the difficulty in balancing task demands between bimodal and two independent unimodal conditions. For example, in the current study, it would have been unfeasible to match the congruency rating judgments required in the bimodal condition by having subjects similarly rate the co-presentation of two odours or two colours. Consequently, calculation of superadditive and subadditive responses in the current study may simply reflect greater attentional demands in the bimodal condition consistent with having to evaluate the congruency of two sensory cues and prepare to make a response. Additionally, according to the principle of inverse effectiveness, superadditive responses are to be expected predominantly when stimuli are presented near threshold level. In this study, both visual and olfactory stimuli were presented well above threshold and under these conditions superadditive effects are not necessarily usual (see Laurienti et al. 2005). For future investigations it may therefore be recommended that stimuli are presented near threshold level. This would also improve the issue of matched odour intensities for the various smells. Even though in the current study, odour intensities have not been rated as significantly different for the group of subjects, it is conceivable that some individuals in the study may have experienced slight intensity differences between certain odours which in turn could have influence the magnitude of the multisensory effects. Therefore, future investigations would preferably present their stimuli at an intensity which is easily comparable between subjects (e.g. threshold level), even though this may necessitate individual intensity calibration for each participant.

Nevertheless, in view of prevalence of these criteria in previous crossmodal imaging studies (Calvert 2001; Foxe et al. 2002; Macaluso and Driver 2005), I have included this additional analysis for comparison purposes. This computation revealed that bimodal activations in the OFC did not only exhibit a linear correlation with subjective odour-colour congruency ratings, but exceeded the sum of the response to either odours or colours presented alone. The identification by both parametric and interaction analyses of the OFC is strongly suggestive of a prominent role for this area in the integration of odour-colour cues. The additional detection, in the superadditive analysis only, of positive bimodal interactions in the anterior cingulate and superior frontal gyrus – areas previously implicated in studies manipulating attention (Badre and Wagner 2004; Francis et al. 1999; Kondo et al. 2004) is more likely to reflect the different task and associated attentional demands between the bimodal and unimodal conditions.

The detection of BOLD responses resembling features of multisensory integration at the neuronal level, suggests that the same mechanism by which multisensory inputs are combined in non-human species – convergence onto sets of bimodal neurones (Stein 1998) – also underlies the integration of colours and odours. That such responses should have been identified in the OFC in the present experiment is consistent with electrophysiological studies of visual-olfactory or olfactory-gustatory integration in non-human primates (Rolls and Baylis 1994) and humans (de Araujo et al. 2003c; Gottfried and Dolan 2003; Small et al. 2004c). It is noteworthy, however, that in the current study there was no evidence of multisensory “response depression” in the presence of incongruent bimodal stimuli (i.e. where the bimodal condition falls below the level of activity elicited by a unimodal stimulus) as has been reported in previous neuroimaging studies of audio-visual (Calvert 2001) and visuo-

tactile integration (Macaluso and Driver 2005). Indeed, studies investigating crossmodal interactions between the chemical senses in humans have also found little or no evidence (de Araujo et al. 2003c; Gottfried and Dolan 2003; Small et al. 2004c) of suppressed responses to incongruent inputs. At the cellular level, this feature of multisensory neurones in the superior colliculus has been shown to be mediated by the presence of inhibitory surrounds which prevent spatially discrepant bimodal stimuli from being co-localized (Stein 1998). This characteristic of multisensory integration however does not appear to be shared by putative multisensory flavour neurones in the OFC.

In a related imaging study by Gottfried & Dolan (2003), olfactory responses in the OFC have also been shown to be modulated by co-presentation of semantically congruent or incongruent pictures associated with odours. Interestingly, as in the current study examining odour-colour interactions, the precise location of these putative intersensory effects within the OFC differed depending on the analytic criteria chosen to identify crossmodal convergence zones. Of note is the observation that the peak activation associated with generic superadditive ($OV > O + V$) responses in the current study, is both bilateral and more medially located than that detected in the odour-picture experiment. However, it does overlap closely with the activation showing a linear correlation with subjective post-scan ratings in the Gottfried & Dolan (2003) study. The corresponding parametric model in the present study (albeit using ratings obtained during rather than post scanning) on the other hand, reveals that the region of OFC sensitive to subjective odour-colour congruency matches lies both lateral and caudal to that implicated in the odour-picture study. Together, these observations would suggest that different visual parameters associated with objects (i.e. colour and features) modulate olfactory-responsive neurones in adjacent OFC

zones that are additionally context-sensitive (i.e. dependent on experimental task requirements).

In contrast to the study by Gottfried and Dolan (2003) the current study failed to implicate a role for the hippocampus in odour-colour integration. Due to our restricted coverage over the frontal third of the brain, we are not able to rule out the possibility that this structure is involved in the synthesis of odours with low-level sensory features such as colour. However, an alternative possibility is that the hippocampus may only play a role in crossmodal binding when mediating the retrieval of semantic associations between odours and pictures – a question for further research.

Finally, previous neuroimaging studies of olfaction suggest stronger engagement of the right OFC during unimodal odour stimulation (Yousem et al. 1999; Zald and Pardo 2000; Zatorre et al. 1992). We observed the same trend towards right OFC activation in the odour alone condition, consistent with this notion. However, we found that modulation of the bimodal responses according to congruency was entirely left lateralized, indicating a functional segregation between the two hemispheres with respect to the type of olfactory task performed. This is consistent with the observation that hedonic judgments of odours elicit brain responses predominantly in the left OFC, whereas judgments of familiarity appear to recruit mainly the right OFC (Royet et al. 2001). Additionally, modulation of the BOLD response according to the perceived pleasantness of liquid flavour stimuli has been found in the left, but not right, OFC (Kringelbach et al. 2003). Lastly, crossmodal superadditive effects and enhancement of activity as a function of bimodal semantic congruency in Gottfried and Dolan's study (2003) were also observed to reside predominantly within the left

OFC. Together, these data suggest that it is specifically the left OFC that integrates olfactory information with visual cues.

I believe the current findings provide a neurophysiological basis for behavioural effects such as changes in the perception of a white wine's odour when it is artificially coloured red (Morrot et al. 2001) and the increased pleasantness of an odour when paired with an appropriate colour (Zellner et al. 1991). A better understanding of how visual cues contribute to our sense of smell and taste could provide insights that will help guide the development of food products, particularly for those with an impaired sense of smell, a problem experienced by as many as 50% of the people over 65 years of age (Schiffman 1997).

8 Multisensory odour-picture integration

The study presented in the previous chapter demonstrated that colour cues could influence olfactory responses in both orbitofrontal and insula cortices, an effect that appeared to be lateralised to the left cerebral hemisphere. In the present chapter, I investigated whether similar effects were also observable using more complex visual stimuli, such as pictures depicting an object. Results revealed a left lateralised network of brain regions comprising of the OFC, hippocampus and piriform cortex/amygdala that responds with increasing strength to more congruent stimuli.

8.1 INTRODUCTION

As already discussed in the previous chapter, visual cues and in particular colours can have a strong influence on various aspects of odour perception, such as intensity and pleasantness perception and odour identification (DuBose et al. 1980; Morrot et al. 2001; Zellner et al. 1991; Zellner and Kautz 1990). There is however less data on how the congruence between an odour and a visual depiction of an odour source (i.e. the smell of apple together with a picture of an apple) may affect the olfactory percept. One of the few electrophysiological investigations on this topic used EEG to record event-related potentials in response to pictures that were preceded by either congruent or incongruent odours. These studies reported difference in the ERP amplitude for the visual N400 waveform when a visual image did not match a previously presented odour for both food related (Grigor 1995) and non-food related

odours (Grigor et al. 1999). Even though both matched and mismatched stimulus pairs elicited a negative peak with a latency of 400 ms after the onset of the visual stimulus, the amplitude of the peak was larger for incongruent stimulus pairs. It should, however, be noted that in this study, odours were used as primes for the pictures and it remains to be shown that similar effects are observable when both stimuli are shown simultaneously.

The first published fMRI study investigating the effect of visual-olfactory congruency was recently conducted by Gottfried and Dolan (2003). In this study pictures and odours were presented either in isolation or as congruent and incongruent odour-picture pairs whilst participants had to detect the presence of an odour. The main result of this study was that congruent stimulus pairs resulted in enhanced neural activity in the left anterior hippocampus and rostromedial orbitofrontal cortex. These findings correspond well with the findings reported in the previous chapter, particularly with respect to a left lateralisation for visual-olfactory integration.

Whilst it appears that olfactory processing per se shows a tendency towards right lateralisation in both primary (piriform) olfactory cortex and in the secondary olfactory cortex located in the orbitofrontal cortex (O'Doherty et al. 2000; Small et al. 1997; Zatorre et al. 1992) such laterality effects depend largely on the type of olfactory task that is performed during scanning. Even though the left OFC has been reported to respond preferentially to aversive odours (Anderson et al. 2003; Zald and Pardo 1997), it has recently been suggested that this region plays a more general role in the emotional processing of odours (Rolls et al. 2003a; Royet et al. 2003) which is not restricted to aversive odours. Judgements of odour familiarity on the other hand have been reported to involve preferentially the right OFC (Royet et al. 2001; Royet et

al. 1999). Based on these findings it is conceivable that the multisensory integration of vision and olfaction may preferentially occur in the left hemisphere.

The study described in the present chapter utilised a similar experimental paradigm as Gottfried and Dolan's (2003), where congruent and incongruent olfactory-visual stimulus pairs were presented to participants whilst they had to detect the presence or absence of an odour. The main difference in the experimental paradigm apart from the use of different odours was the fact that congruency judgements were obtained immediately after each stimulus presentation rather than after the experimental session. This allowed for a more precise modelling of the height of the BOLD response to each of the presented stimulus pairs. Additionally, only bimodal trials were presented in the present study because of the interpretative problems associated with the superadditivity analysis as discussed in the previous chapter. I hypothesised that a left lateralised network of areas in OFC and insula cortex that has previously been shown to be involved in colour-odour integration (Chapter 7) as well as the hippocampal and OFC regions shown to respond more strongly to congruent odour-picture pairs by others (Gottfried and Dolan 2003) would show modulation of the BOLD signal according to the perceived odour-picture congruency.

8.2 METHODS

8.2.1 Participants

Twelve healthy right-handed volunteers participated in this study (7 females and 5 males; mean age 26 years, age range 22-31 years). Each participant was scanned twice with the same functional paradigm, but with a 'mouthshim' fitted during one of the scanning sessions as described in Chapter 4. In order to control for session effects, half of the participants were scanned with the 'mouthshim' in the first session. The first 30 functional EPI volumes of one subject (in the with 'mouthshim' condition) had to be discarded from the study because of excessive motion during the first 1 ½ minutes of scanning so that data analysis at the group level includes one truncated dataset. Additionally, during 2 of the experimental runs, a technical error prevented the collection of the rating responses, so that the group analysis includes a total of 22 datasets. The study was approved by the Central Oxford Research Ethics Committee (C99.179).

8.2.2 Stimuli and task

During scanning, subjects were presented for 6 s with visual-olfactory stimulus pairs, followed by a rest period of 30s, during which only clean air was delivered to the subjects. A total of seven different olfactory stimuli were used for this study (apple, spearmint, orange, banana, butyric acid, galbanum oil and strawberry; see Table 8.1), all provided by Quest International. Odours were diluted in 50ml of diethyl phthalate (CAS number 84-66-2) which itself has minimal odour and dissolves a wide range of odorants. Digitised photos depicting objects that are associated with the various odours were used as visual stimuli (picture of apples, toothpaste, oranges, bananas, cheese, forest and strawberries).

	<i>Orange</i>	<i>Apple</i>	<i>Banana</i>	<i>Strawberry</i>	<i>Cheese</i> <i>(Butyric acid)</i>	<i>Spearmint</i>	<i>Galbanum</i>
Concentration	15%	10%	10%	5%	0.1%	20%	1%
Picture							

Table 8.1: Olfactory and visual stimuli.

The table shows the concentration of each odour in diethyl phthalate as well as their associated congruent pictures.

Each odour was presented twice together with a congruent picture (e.g. smell of apple with the picture of apples) and twice with 2 randomly chosen incongruent pictures (e.g. smell of apple with the picture of toothpaste and with the picture of strawberries), resulting in a total of 28 stimulus presentations. The order of the presentations was randomised for each participant, with the condition that the same odours were never presented consecutively. All olfactory stimuli were delivered with a custom-built, computer-controlled olfactometer. Subjects were asked to breathe normally through their nose while an air-stream of either odourised or odourless air was delivered at a flow rate of 6 litres/second through a Teflon tube placed directly under the subject's nose. Full screen colour images were generated using a video projector located outside the scanner room and projected onto a translucent screen placed directly outside the magnet bore. Subjects wore prism glasses so that they could see the screen while lying in the scanner. The task during the scanning was firstly to detect both onset and offset of each olfactory stimulus and secondly to rate each picture-smell combination with respect to 'goodness of fit between the picture and smell' on a rating scale ranging from 1='very good' to 4='very bad'. Subjects indicated both measures by pressing one of 4 buttons. Behavioural measures relating to the congruence between pictures and odours were collected 15s after the end of each bimodal stimulus presentation, when subjects were visually cued to with the word 'RATE' on the screen to enter their rating.

8.2.3 Data acquisition and analysis

Both functional and structural MRI images were acquired using a 3T Varian Inova spectrometer (Varian Inc. Palo Alto, CA) fitted with a Magnex SGRAD head

gradient coil (Magnex Scientific Ltd., Oxfordshire, UK) and a birdcage transmit/receive head coil. For each of the functional data series, a total of 340 T2* weighted echo-planar imaging (EPI) volumes were taken over a time period of 17 min. Each volume consisted of 24 continuous oblique (tilted approximately 10° upwards from the anterior to posterior commissure line, so as to be aligned with the temporal lobes) slices of 3mm thickness with an in-plane resolution of 4×4mm. These imaging parameters allowed for imaging the ventral two thirds of the brain until approximately Z-coordinate of +50 of the MNI 152 standard space, to include all primary and secondary olfactory areas in orbitofrontal cortex and temporal lobes as well as the visual cortex. Other imaging parameters were: TR=3s, 64×64 matrix, FOV 256×256 mm, TE=30ms, interecho spacing $\Delta t = 746\mu\text{s}$, flip angle = 90° and readout bandwidth = 100 kHz. Additionally, an automated global shimming method was used (Wilson et al. 2002a). After each of the two experimental sessions, a B₀ field map was acquired using a combined symmetric and asymmetric spin echo sequence.

For registration into standard space, a whole brain T2* weighted EPI volume (54 slices, TR=7s, other imaging parameters as above) and a high-resolution, whole-brain T1 weighted morphological scan (inversion-recovery fast gradient echo, 1.5mm slice thickness, 1.5mm×1.5mm in-plane resolution) were acquired after the experimental paradigm.

Statistical image analysis of the functional dataset was carried out using FEAT, the FMRI Expert Analysis Tool (www.fmrib.ox.ac.uk/fsl). Prior to data analysis the first 3 volumes were deleted (to avoid intensity changes due to the approach to steady state magnetization) and the following pre-processing was applied: motion correction using MCFLIRT (Jenkinson et al. 2002); spatial smoothing using a Gaussian kernel of FWHM 5mm; mean-based intensity normalization of all volumes

by the same factor; non-linear high-pass temporal filtering (Gaussian-weighted LSF straight line fitting, $\sigma=36.0s$). A general linear model (GLM) using the timing of the 28 bimodal odour-picture presentations was fitted to the time course at each voxel. A second explanatory variable was used to model the parametric variation of the BOLD response with the perceived congruency of the odour-picture pairs as indicated by the subject's button presses during the experiment. Statistical analysis for each experimental run was carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich et al. 2001).

For group analysis, the individual results were registered both to high-resolution anatomical MR images and to the Montreal Neurological Institute (MNI) 152 standard image. Registration to high resolution and standard images was carried out using FMRIB's Linear Image Registration Tool (FLIRT) (Jenkinson et al. 2002). The statistical analysis focused on the parametric variation of the BOLD response with the perceived congruency of the odour-picture pairs. Mixed-effects (often referred to as 'random-effects') group analysis was carried out using FMRIB's Local Analysis of Mixed Effects (FLAME) software (Beckmann et al. 2003) with a cluster threshold of $Z>2.0$ and a cluster significance threshold of $P=0.05$ (corrected for multiple comparisons) (Forman et al. 1995; Friston et al. 1994; Worsley et al. 1992).

8.3 RESULTS

8.3.1 Behavioural

The analysis of the behavioural responses for the perceived congruency of the stimuli demonstrated that the subjects could clearly distinguish between the congruent and incongruent odour-picture pairs. All differences between the ratings of the congruent and the incongruent stimulus pairs were highly significant (paired t-test). The stimulus pair rated as matching best of all the congruent stimuli was that of apple and that rated the worst was that of strawberry (Table 8.2).

	<i>Orange</i> <i>Mean±SD</i>	<i>Apple</i> <i>Mean±SD</i>	<i>Banana</i> <i>Mean±SD</i>	<i>Strawberry</i> <i>Mean±SD</i>	<i>Cheese</i> <i>Mean±SD</i>	<i>Spearmint</i> <i>Mean±SD</i>	<i>Galbanum</i> <i>Mean±SD</i>
Congruent	1.3 ± 0.5	1.1 ± 0.2	1.9 ± 0.9	2.9 ± 0.8	2.3 ± 1.2	1.7 ± 0.9	1.6 ± 0.9
Incongruent	2.9 ± 0.8	2.4 ± 0.9	3.4 ± 0.8	3.3 ± 0.7	3.6 ± 0.7	3.3 ± 0.7	3.6 ± 0.5
Paired	T = 13.8	T = 9.6	t = 9.3	t = 2.8	t = 6.0	t = 10.0	t = 14.1
t-test	p<0.001	P<0.001	p<0.001	p<0.01	p<0.001	P<0.001	p<0.001

Table 8.2: Odour-picture congruency ratings.

The table shows the ratings for congruency for all 7 odours presented together with either a matching or a mismatching picture (n=22) on a scale from 1=very good match to 4=very bad match. Paired t-tests confirmed that the a priori assumed congruent stimulus combinations were indeed perceived as significantly better matching than the mismatching ones for all odours.

8.3.2 Bimodal olfactory-visual activation

Consistent with previous studies of the olfactory and visual system, a network of areas including the frontal and orbitofrontal cortices, insula cortices, anterior cingulate, basal ganglia, primary visual cortex and medial temporal regions were

activated (see Table 8.3) by the simultaneously presented olfactory-visual stimuli. In the frontal and OFC regions only, the bimodal stimuli elicited stronger activation (as indicated by higher z-scores) in the right hemisphere. This was particularly pronounced in the medial orbital gyrus that only showed significant activation in the right hemisphere (Figure 8.1).

<i>Brain region</i>	<i>Side</i>	<i>MNI coordinates</i>	<i>Z-score</i>
Lateral orbital gyrus	R	40/52/-15	5.1
	L	-26/51/-19	2.9
Medial frontal gyrus	R	44/46/0	6.3
	L	-45/56/-1	4.9
Medial orbital gyrus	R	23/41/-23	3.2
Inferior frontal gyrus	R	44/34/28	6.4
	L	-44/33/27	5.5
Anterior cingulate	R	12/17/32	4.8
	L	-8/18/30	4.6
Caudate	R	18/12/15	4.7
	L	-18/14/10	4.7
Insula cortex	R	46/18/-4	5.0
	L	-38/17/1	5.2
Amygdala / piriform cortex	R	29/2/-20	3.2
	L	-36/5/-22	3.2
Pallidum	R	19/-2/-3	4.2
	L	-17/-3/-6	3.8
Thalamus	R	16/-14/16	3.9
	L	-18/-14/12	4.2
Hippocampus	R	28/-20/-12	4.5
	L	-32/-13/-15	4.2
Supramarginal gyrus	R	56/-42/32	4.7
	L	-58/-44/30	4.4
Lingual gyrus	R	18/-72/2	5.3
	L	-6/-76/2	6.0
Striate cortex (BA 17)	R	10/-88/-4	5.0
	L	-4/-88/-4	5.9

Table 8.3: Visual-olfactory activation.

Brain areas activated by the bimodal olfactory-visual stimuli in the MNI 152 standard space. The depicted values correspond to the highest activated voxel within each cluster (n=22).

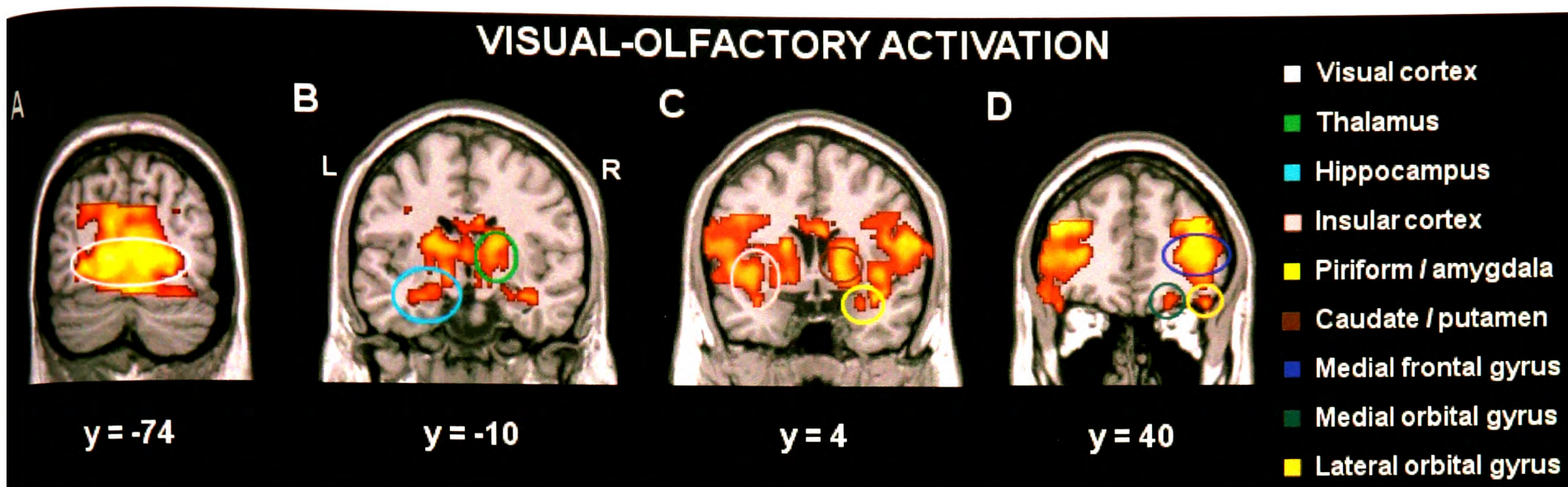


Figure 8.1: Bimodal picture-odour activation.

Group activation map ($n=22$) for olfactory-visual activation independently of their congruency ratings. **A** Extensive activation was detected in primary visual cortex. **B** Bilateral activation was found in the hippocampus and thalamus as well as in **C** the insula cortex, basal ganglia and the piriform cortex and adjoining amygdala. **D** Activation in frontal brain regions was found bilaterally in the medial frontal gyrus and lateral orbital gyrus, whereas activation in medial orbital gyrus was lateralised to the right hemisphere. The right side of each slice corresponds to the right side of the brain.

8.3.3 Multisensory olfactory-visual integration

To reveal brain areas sensitive to the crossmodal congruency between the visual stimulus and co-presented odour, I investigated areas that showed a correlation between the BOLD signal and the behavioural ratings of congruency on a trial by trial basis. This parametric analysis revealed a predominantly left lateralised network of brain areas comprising of the hippocampus and parahippocampal gyrus ($x = -26$, $y = -22$, $z = -18$; $z\text{-score} = 3.26$), amygdala/piriform cortex ($x = -26$, $y = 0$, $z = -18$; $z\text{-score} = 3.18$) and the caudal OFC ($x = -30$, $y = 26$, $z = -20$; $z\text{-score} = 2.62$) that exhibited increasingly stronger responses to progressively higher perceived congruency (Figure

8.2). Additionally, a small cluster of activation was detected in the right parahippocampal gyrus ($x = 22, y = -22, z = -20$; $z\text{-score} = 2.48$).

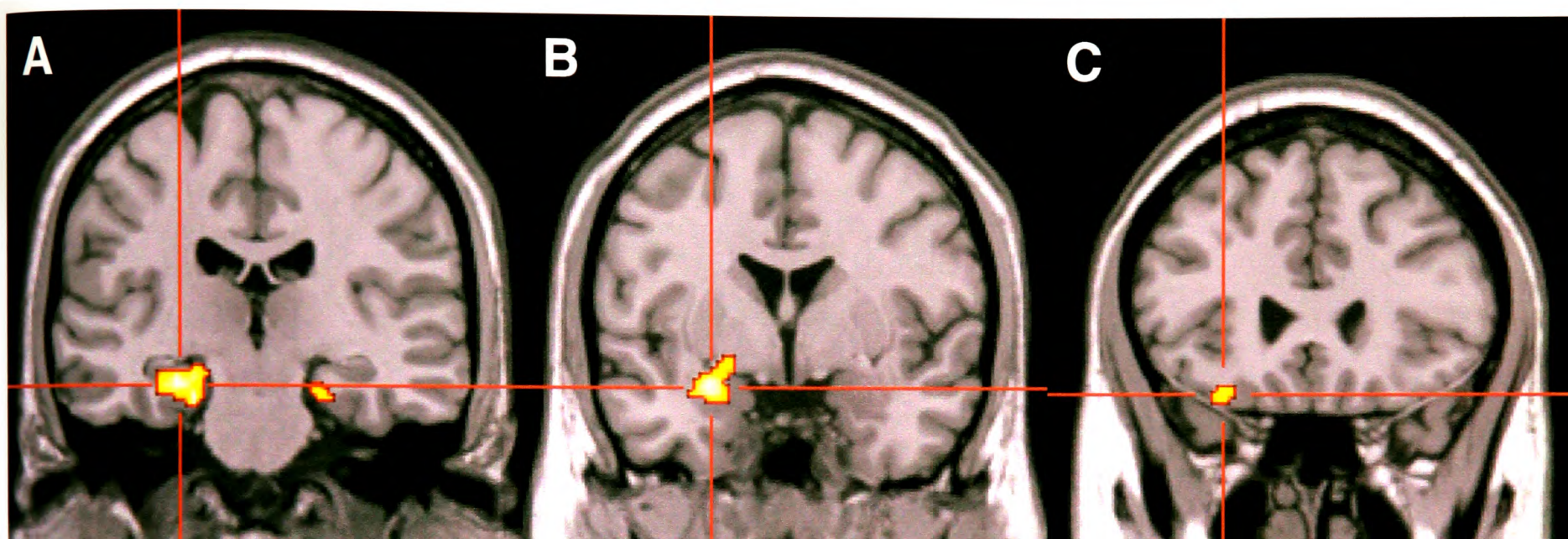


Figure 8.2: Modulation of the odour responses according to congruency.

The activated areas in **A** left hippocampus and parahippocampal gyrus ($x = -26, y = -22, z = -18$; $z\text{-score} = 3.26$) and right parahippocampal gyrus ($x = 22, y = -22, z = -20$; $z\text{-score} = 2.48$) **B** left amygdala/piriform cortex ($x = -26, y = 0, z = -18$; $z\text{-score} = 3.18$) and **C** left caudal orbitofrontal cortex ($x = -30, y = 26, z = -20$; $z\text{-score} = 2.62$) displayed increasingly stronger BOLD signals in response to increasingly higher perceived congruency of the odour-picture pairs. The right side of each slice corresponds to the right side of the brain.

A detailed analysis of the percentage BOLD signal change established that the various regions of the network displayed slightly different responses to the stimuli (Figure 8.3). The highest BOLD signal changes could be observed in the OFC and this region appeared to respond particularly strongly when the congruency between odour and picture was very high, whereas responses for lower congruency ratings were not significantly different from each other. Responses in medial temporal lobe structures (piriform / amygdala and hippocampus / parahippocampal gyrus) were

varying linearly with the perceived congruency and were very similar in magnitude. Interestingly, stimuli with very low perceived congruency elicited a BOLD response very close to zero which could be indicative of neural suppression effects in these brain regions.

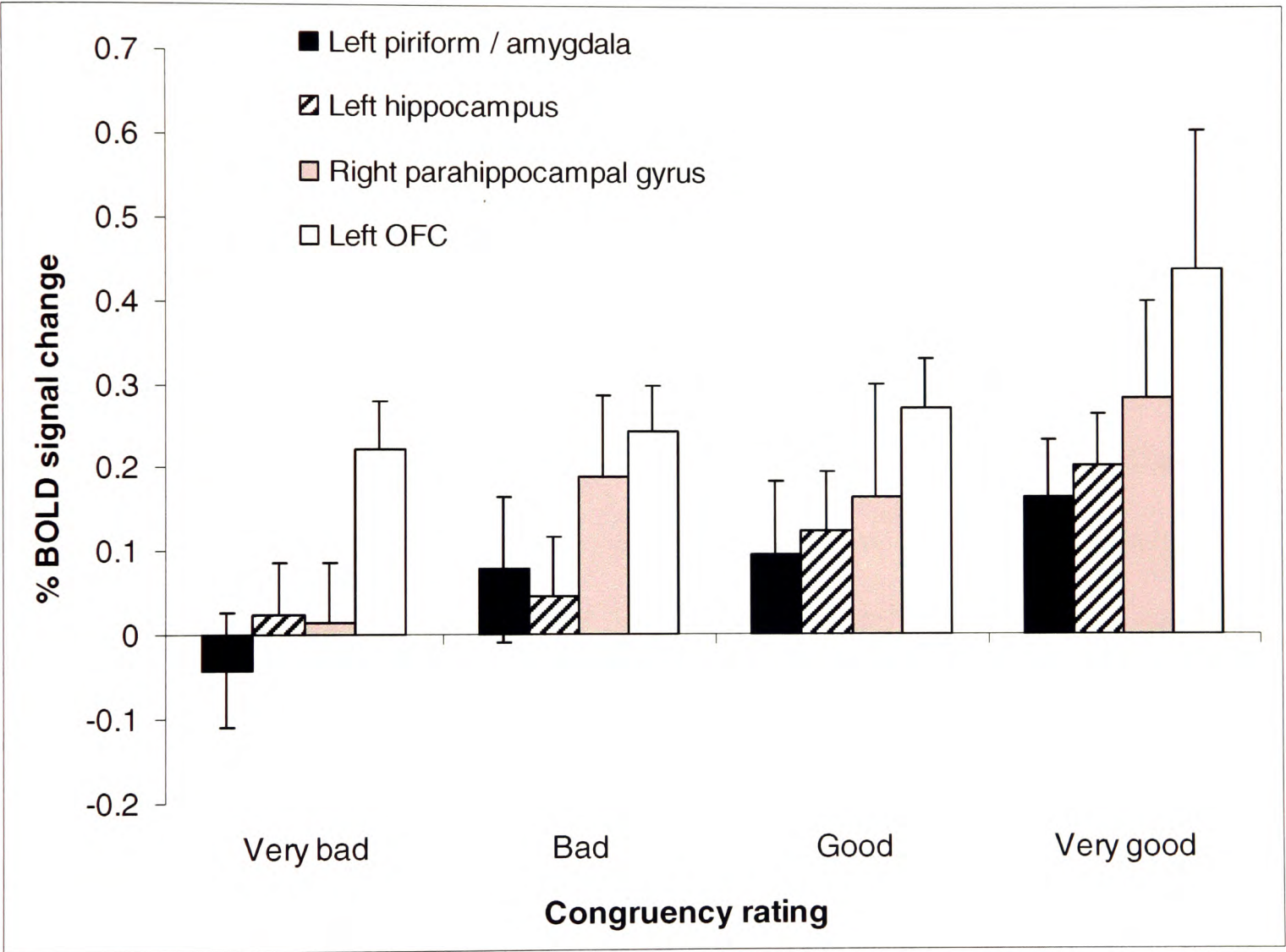


Figure 8.3: BOLD signal changes.

Percentage BOLD signal changes according to the perceived congruency in all brain areas displaying congruency effects (n=22). Error bars indicate the standard error of the mean (SEM).

8.4 DISCUSSION

The aim of the present study was to investigate cerebral responses to odour-picture pairs of varying congruency. Statistical analysis of the behavioural data confirmed that all stimulus pairs which were a priori classified as congruent (i.e. the picture of an orange together with the smell of oranges) were indeed perceived as significantly more congruent by our participants than the same odours together with a random selection of the other pictures (i.e. the smell of oranges with a picture of strawberries). The highest perceived congruency was observed for the apple odour-picture pair and the lowest for that of strawberry. It appears unlikely that the low perceived congruency for the strawberry odour compared to the apple odour is simply a function of familiarity with this smell, since both odours were rated as being of virtually identical familiarity (5.28 for apple and 5.25 for strawberry) in a previous behavioural study (see Chapter 6). However, the majority of subjects in this previous study used descriptors such as ‘artificial strawberries’, ‘bubble gum’, ‘strawberry bath bomb’ or ‘strawberry sweets’ for this odour and it appears that the particular strawberry odour used in this study was more reminiscent of such strawberry flavoured products rather than actual strawberries, which could explain the low congruency ratings for this odour when presented together with a picture of strawberries. A similar effect could be responsible for the relatively low perceived congruency between the odour butyric acid and pictures of cheese. Even though this chemical has a cheese-like aroma it does not reflect the multitude of odours present in actual cheese since it is a single compound. It should however be noted that the ratings for each stimulus presentation were used in the parametric analysis of the fMRI dataset so that such differences between the various congruent stimuli were accounted for.

Independently from their congruency ratings, the visual-olfactory stimulus pairs elicited activation in both piriform and striate cortex, corresponding to the primary olfactory (Zatorre et al. 1992) and visual (Tootell et al. 1998) cortices respectively. Two other temporal lobe regions in close proximity to the piriform cortex were also responsive to the stimulation, notably the amygdala and hippocampus. Whilst amygdala activation is frequently reported in olfactory neuroimaging studies (Zald and Pardo 2000) and appears to encode both the valence and intensity of the odour stimulus (Anderson et al. 2003; Gottfried et al. 2003; Winston et al. 2005) hippocampal activation is reported less often (Poellinger et al. 2001; Wang et al. 2005) and may predominantly reflect memory encoding and retrieval associated with the stimulus pairs as discussed below.

Secondary olfactory regions in the medial and lateral OFC as well as in the insula cortex and anterior cingulate were also found to be activated by the odour-picture stimuli irrespective of their congruency. Both regions have frequently been reported in neuroimaging studies using odour stimuli (Small and Prescott 2005; Zald and Pardo 2000) and the insula cortex has been previously been shown to be involved in the multisensory integration of odour and taste stimuli (de Araujo et al. 2003c; Rolls 2004c; Small et al. 2004b). Even though activity in parts of the anterior cingulate have been found to correlate with the subjective pleasantness of odour stimuli (Rolls et al. 2003a), activation in this region may at least partly reflect neural activation associated with performing the task of judging congruency.

Consistent with previous brain imaging studies on human olfaction, there was a trend towards right lateralisation in olfactory areas within the OFC (Royet et al. 2001; Zatorre et al. 1992). Activation in inferior frontal gyri has also been reported in several previous neuroimaging experiments on olfaction (Plailly et al. 2005; Royet et

al. 1999) and activation in the left hemisphere has been linked to higher order olfactory judgements such as the familiarity and edibility of the presented odours. However, in the present study, activity in frontal brain regions was observed bilaterally with a tendency of slightly stronger activation in the right hemisphere which may indicate that effects of hemispheric dominance in these brain regions are dependent on the particular experimental task.

Of primary interest in this study were brain regions sensitive to the perceived congruency between the odours and pictures which would indicate their involvement in the sensory integration between the two modalities. Several such regions were identified, where the height of the BOLD response varied according to the perceived congruency, namely the piriform cortex and adjoining amygdaloid complex, the orbitofrontal cortex and the hippocampus/parahippocampal gyrus.

Interestingly, our data suggests that visual inputs can already modulate neural responses in primary olfactory cortex (POC). This brain region displayed no appreciable response to incongruent odour-picture pairs but responded increasingly stronger with higher congruency. Even though the experimental design did not allow one to directly assess effects of neural suppression, the fact that only odours accompanied by a congruent picture elicited a response in POC suggests that incongruent visual inputs may suppress the odour response in this region. This interaction in POC between the two modalities may well help to explain the behavioural findings where appropriately coloured liquids are perceived as more intense than inappropriately coloured ones (Zellner and Kautz 1990) and the colour of a wine influences its perceived aroma (Morrot et al. 2001), since odour intensity and quality appears to be encoded in this brain region (Anderson et al. 2003; Gottfried et al. 2006).

The OFC is another region that is recruited in virtually any olfactory task, as demonstrated by the fact that all neuroimaging studies concerning olfaction report activation in this region (see (Rolls 2004b; Small and Prescott 2005; Zald and Pardo 2000) for reviews). In contrast to the findings of Gottfried and Dolan (2003) who reported interactions between odours and pictures in rostromedial OFC and the findings of the preceding chapter, interactions between the two modalities were found in the left caudolateral OFC in this study. Interestingly, the OFC site of interaction in the present study is located in a region that shows significantly improved B_0 homogeneity in OFC due to the use of the ‘mouthshim’ (see Chapter 4) whereas the rostromedial OFC reported by the other studies lies within the area of the susceptibility artefact and hence low BOLD sensitivity. Additionally, the caudolateral OFC region reported here lies in close proximity to the area that Gottfried and Zald (2005) have recently identified in their meta-analysis of 13 olfactory neuroimaging studies as the primary site for olfactory representations within the OFC and has also been reported to be involved in hedonicity judgements of odours (Royet et al. 2001). As such, it is conceivable that both areas are involved in visual-olfactory integration but detection depends at least to some extent on the particular imaging protocol utilised.

Similar to the findings of Gottfried and Dolan (2003) the hippocampus displayed a modulation according to perceived odour-picture congruency. These activations are likely to reflect retrieval processes of previously established associations between an odour and a corresponding object that emanates this particular smell (Eichenbaum et al. 1996). Further evidence for this assumption is provided by the observation that hippocampus and parahippocampal regions show significantly greater responses to personally significant odours than to similar cues in

other sensory modalities (Herz et al. 2004). The active retrieval of previous odour-object associations would indeed be necessary to perform the task during the experiment, which required assessing the degree to which odour and picture were matching. It is therefore likely that activation in this region is the neural correlate of accessing previously established odour associations.

With the exception of a small cluster in the right parahippocampal gyrus, all activation was located in the left cerebral hemisphere, a finding which corresponded well with that obtained when a colour-odour matching task was performed (Chapter 7) and also reported for congruent odour-picture combinations (Gottfried and Dolan 2003). In contrast to these observations, the finding of two recent studies suggest that tasks of olfactory recognition memory preferentially activate medial temporal lobe structures in the right hemisphere (Cerf-Ducastel and Murphy 2006; Gottfried et al. 2004). It should however be noted that the activation observed in these studies was elicited by either the names or pictures that had been associated with a variety of smells and no odours were presented during the scanning sessions. In the present study, odours and pictures were co-presented and their semantic congruency had to be evaluated by the participants. Additionally, a series of lesion and PET studies conducted by Dade and colleagues (2002) concluded that olfactory recognition memory required inputs from both hemispheres. It has also been reported that any effects of lateralisation are largely dependent on the specific odour judgements (such as familiarity, edibility or pleasantness) performed (Plailly et al. 2005; Royet et al. 2001; Royet et al. 1999; Royet et al. 2003), so that no general hemispheric superiority exists for the performance of olfactory tasks. Taken together, the results of the present study and those found by others (Gottfried and Dolan 2003) suggest however that

multisensory integration of odours occurs preferentially within the left side of the brain.

9 Conclusions and future directions

The primary aim of this thesis was to investigate processes of multisensory integration involving the sense of smell with fMRI. To achieve this goal, two technical challenges had to be addressed: the ability to obtain MR images of sufficient quality in olfactory brain regions within the orbitofrontal cortex and the construction of a stimulus delivery system adequate for rapid and controlled odour delivery in the MR environment. Two possible methods for improving OFC image quality have been investigated: sensitivity encoding and passive shimming. Furthermore, two methods for olfactory stimulus delivery using either an air-flow olfactometer or odours dissolved in liquid were investigated. Finally, the mechanisms of visual-olfactory integration were investigated both behaviourally and using fMRI. In this chapter, the conclusions from these studies and directions for future research are discussed.

9.1 IMAGING THE HUMAN ORBITOFRONTAL CORTEX

As the OFC is the primary brain area involved in visual-olfactory integration (Gottfried and Dolan 2003; Rolls 2004a; 2001; Rolls and Baylis 1994), obtaining high quality MR images in this region is a necessary requirement for any fMRI investigation concerned with the study of the chemical senses. As detailed in Chapter 2, the presence of susceptibility artefacts in the OFC constitutes a major challenge for such investigations, particularly when images are acquired at high field strength

(Abduljalil and Robitaille 1999; Krasnow et al. 2003). Consequently, two studies reported in this thesis explored methods to improve the signal originating from the ventral section of the frontal lobe.

In the first of these studies (Chapter 3) single-shot spiral imaging using sensitivity encoding (SENSE) (Pruessmann et al. 2001; Pruessmann et al. 1999) was compared to conventional spiral acquisition in a gustatory activation paradigm. Results confirmed that the signal-dropout in OFC was reduced during SENSE acquisition, albeit at the cost of a reduced SNR across the entire brain. For investigations particularly concerned with OFC function, such reductions in overall SNR may well be compensated for by the reduced signal loss and geometric distortions in OFC during SENSE acquisition.

Even though group analysis of the functional activation maps has to be treated with caution because of the small sample size (5 subjects), the results also demonstrated a larger number of activated voxels during the SENSE acquisition. Surprisingly, conventional spiral imaging resulted in detectable activation in the inferior OFC which was not observed using SENSE acquisition. However, this activation was located well within the frontal susceptibility artefact and it cannot be ruled out that it is at least partly caused by stimulus-correlated motion rather than neural activity. Conversely, bilateral activation in the primary taste cortex located in the anterior insula and adjoining frontal operculum (Rolls 2005; Small et al. 1999a) was only detected using SENSE.

Overall spiral imaging using SENSE may prove to be a feasible alternative to gradient echo methods for imaging the human OFC, particularly at higher field strength. Additionally, the current constraints for using this technique, in particular the fact that SENSE acquisition is not routinely implemented in MR scanners and the

high computational demands for image reconstruction, may well be removed in the future.

An alternative method for enhancing image quality in OFC was investigated in a second fMRI experiment (Chapter 4). This involved improving field homogeneity passively by placing a highly diamagnetic material into the mouth of participants ('mouthshim'), as suggested by Wilson and colleagues (2002b; 2003). In a bimodal olfactory-visual stimulation paradigm, the same set of participants was scanned both with and without the 'mouthshim'.

Wearing the 'mouthshim' had a significant effect on head motion, resulting in increased rotational motion around the x-axis and translational motion along the z-axis when participants were wearing the device. This increased motion was attributed to the observation that the placement of such a device into the mouth increased salivary flow, which in turn resulted in more frequent swallowing movements. Even though this motion did not appear to have a detrimental effect on functional activation in OFC, this problem could be minimised by incorporating the device into a bite-bar.

Importantly, wearing the 'mouthshim' resulted in significantly stronger BOLD responses within the OFC. This effect was most likely due to the increased field homogeneity caused by the device which was demonstrated by comparing the B_0 field maps obtained during both experimental sessions. Whilst the average field homogeneity across the entire brain was not significantly different, wearing the mouthshim improved B_0 homogeneity approximately by a factor of five in OFC resulting in a 14% increase in BOLD sensitivity. Further analysis of the field gradients in this region revealed that even though the phase encoding gradients (y) were lowered by the 'mouthshim', these improvements were predominantly due to a decrease of the in-plane x-gradients.

Surprisingly, wearing the ‘mouthshim’ decreased activation in insula cortices. A detailed analysis of the B_0 field homogeneity and BOLD sensitivity ruled out the possibility that these decreases were due to the physical properties of the device, because field indices were either not different or slightly improved by wearing the ‘mouthshim’. It seems therefore more likely that these effects in insula cortices have a physiological origin associated with having a foreign object placed in the mouth. As reported by the participants and indicated by the motion analysis, wearing the ‘mouthshim’ increased salivary flow.

A further possibility is that participants may have explored the device with their tongues during the experimental session, resulting in increased tongue movements. Since swallowing as well as somatosensory and motor stimulation of the tongue are known to engage insula cortex (Cerf-Ducastel et al. 2001; Fesl et al. 2003; Furlong et al. 2004; Harris et al. 2005; Martin et al. 2004; Miyamoto et al. 2006; Toogood et al. 2005), this may have lead to increased BOLD signals during the rest periods of the experimental session, leading in turn to a decrease in the magnitude of BOLD contrasts when the rest periods were subtracted from the stimulation periods in the functional analysis. To a certain extent, these effects could be minimised by either monitoring swallowing or alternatively by giving participants cues when they are allowed to swallow, so that swallowing can be explicitly modelled as a variable of no interest. Additionally, participants could be instructed to refrain from exploring the ‘mouthshim’ with their tongues, thereby minimising the somatosensory and motor components. Nevertheless, it is not recommended to use the ‘mouthshim’ for fMRI experiments that are primarily concerned with activation in insula regions, such as gustatory investigations (de Araujo et al. 2003a; Rolls 2005; Small et al. 1999b; Zald et al. 2002).

Overall, the ‘mouthshim’ has proven to be a useful device for improving image quality in OFC and its use is therefore recommended for fMRI studies primarily concerned with OFC function. Combining the ‘mouthshim’ with other methods such as specialised pulse sequences, gradient compensation and optimised slice angles (Deichmann et al. 2003; Li et al. 2002; Ojemann et al. 1997; Posse et al. 2003) may then potentially enable a researcher to observe neural activation in regions that would otherwise be lost due to the susceptibility artefacts.

9.2 OLFACTORY STIMULUS DELIVERY

The delivery of odorants during an fMRI experiment constitutes a further methodological challenge as only one MR-compatible olfactometer is commercially available which was developed by Kobal (1985) and is produced by Burghard (Wedel, Germany). As already mentioned in the Methods section of this thesis, this olfactometer could not be used because of its high cost. Furthermore, the current system provided by this company only allows for the birhinal presentation of 3 different odours, whereas one of the studies presented here used 7 different odours. Therefore, as reported in Chapter 2, the construction of an air-dilution olfactometer suitable for the MR environment was undertaken. This olfactometer allowed for computer-controlled delivery of either odourless or odourised air in the absence of any auditory or tactile cues that could have alerted participants to a switch of the system between the two.

However, the requirement to ‘probe’ the air-stream for the presence of odours in all subsequent functional experiments reported in the thesis, meant that stimulus-correlated motion was an unavoidable drawback of using this set-up.. Because

olfactory stimulation using this method is time-locked to respiration, artefactual activation due to associated head motion could have been a potential confound. Indeed, sniffing odourless air in itself has previously been demonstrated to cause neural activity in olfactory brain regions (Mainland and Sobel 2006; Sobel et al. 1998a).

To investigate the potential influence of such stimulus-correlated motion and sniff-related neural activity on fMRI activation maps, a pilot study was carried out which required participants to take a deep breath following an auditory cue. The results demonstrated that the motion caused by these inhalations did indeed result in a certain level of motion artefact in the fMRI data, particularly along the ventricles. Such motion artefacts are commonly observed around the ventricles, since the signal intensity differences between CBF and white matter are the most pronounced there. Importantly, neither motion artefacts nor sniff-induced activation could be observed in regions of primary and secondary olfactory cortices (Anderson et al. 2003; Gottfried et al. 2002b; Rolls 2001). In view of this observation, the fMRI experiments described in this thesis were conducted with freely breathing subjects, rather than using the technique of velopharyngeal closure (Kobal 1985). Even though this technique has the advantage of completely eliminating stimulus correlated motion, it was not employed for the studies described in this thesis for two reasons. Firstly, it decreases participant's comfort during scanning, since a tube has to be inserted into their nasal passage. More importantly, the air-stream delivered by the olfactometer has to be at physiological levels with respect to humidity (close to 100%) and temperature (around 36°C), because failure to do so would result in rapid drying out and irritation of the nasal epithelium which clearly is not desirable for investigations on olfaction. Providing such a heated and humidified air-stream constitutes a further technological

challenge, since the entire tubing system would require to be heated to prevent condensation within the tubing (which would result in droplets of water being sprayed onto the olfactory epithelium a participant).

A second stimulus delivery system, allowing for the delivery of odours dissolved in a liquid (Cerf-Ducastel and Murphy 2001; de Araujo et al. 2003a; Kringelbach et al. 2003) was implemented for the gustatory-based studies described in this thesis. Essentially, this system allows an experimenter to inject a given quantity of liquid into the mouth of a participant (see Chapter 2 for details). A study comparing two different experimental designs, either swallowing immediately after stimulus delivery or holding the stimulus in the mouth for 9s is described in Chapter 3. Since many of the behavioural studies describing visual-olfactory interactions have been carried out using either food or drinks (Clydesdale 1994; 1993; King and Duineveld 1998; Moir 1936; Pangborn et al. 1963; Pangborn and Hansen 1963b; Rolls et al. 1982), a method for investigating similar interactions in the fMRI scanner was deemed to be highly relevant. Furthermore, as several of the colour-odour associations studied so far involved edible items (Cardello and Sawyer 1992; Zellner and Kautz 1990; Zellner and Whitten 1999), especially fruits that have a characteristic colour (i.e. banana and yellow), I predicted that, multisensory effects may be stronger when odours are presented retronasally (Rozin 1982; Small et al. 2005), where the olfactory component reaches the olfactory epithelium via the mouth rather than via the nostrils.

In previous fMRI studies using flavour stimuli, participants were required to hold the stimuli in their mouths for approximately 10s before swallowing it (de Araujo et al. 2003a; Kringelbach et al. 2003; O'Doherty et al. 2001; Small et al. 2003). Whilst such an experimental design has the advantage of minimising stimulus-correlated motion, it also reduces the quantity of volatiles reaching the olfactory

epithelium thereby biasing stimulation towards the gustatory modality, since volatile release is associated with mouth movements and swallowing (Buettner et al. 2001; Burdach and Doty 1987; Burdach et al. 1984). Consequently, the primary goal of the study described in Chapter 3 was to investigate whether it was feasible to have participants swallow a liquid immediately after its delivery and thereby increase the olfactory components of the flavour percept.

Using a sweet banana-flavoured liquid, and a control solution consisting of KCl and NaHCO₃ dissolved in distilled water in ionic concentrations thought to mimic those found in human saliva (de Araujo et al. 2003a; Kringelbach et al. 2003; Small et al. 2003), two different experimental designs were investigated in the same population of participants. In one condition, participants were required to hold the liquids in their mouth for 9s ('hold' condition) whereas in the other condition they were required to swallow the liquid immediately after delivery ('swallow' condition).

Several findings emerged from this study. Firstly, swallowing the stimulus caused a significant increase in stimulus-correlated head motion. Even though no strong motion artefacts were observed at the border of ventricles and the majority of cortical regions, negative BOLD changes were observed in frontal- and orbitofrontal cortex. Since these are more likely to be caused by partial volume effects rather than functional deactivation, this finding indicates that the head motion induced by swallowing has a detrimental effect on signals originating from OFC. A reduction in such motion could possibly be achieved by the use of a bite bar, thereby minimising this problem.

Of some surprise was the observation that the control liquid caused activation in areas identical to those activated by the flavour stimulus. Several factors may have contributed to this finding. Firstly, the slightly salty nature of the control solution (as

reported by the participants) is likely to have engaged gustatory brain areas. Therefore, it may be desirable for future investigations to utilise a solution more closely resembling that of human saliva in taste, temperature, viscosity and texture. Secondly, previous studies have shown that tongue movements, swallowing and somatosensory stimulation of the oral cavity result in neural activation in brain regions shared with gustatory and flavour processing (Furlong et al. 2004; Harris et al. 2005; Martin et al. 2004; Small and Prescott 2005). It therefore appears that neurones processing olfactory, gustatory and somatosensory inputs are interspersed in the same cortical regions, particularly within the insula cortex. Consequently, flavour related activation after subtraction of the control solution is relatively small. Other than adopting a region of interest analysis which allows for a higher statistical threshold (and therefore detectability of smaller differences), there is no solution to this problem.

Despite the considerable activation elicited by the control solution, differential patterns of activation were nonetheless identified between the flavour and control stimuli in primary and secondary olfactory and gustatory cortices. Whilst both experimental paradigms activated piriform cortex and adjoining amygdala, as well as the insula cortex, OFC activation was only observed in the 'hold' experiment. This is a serious concern for multisensory integration studies involving the chemical senses, since the OFC is a primary site for investigation (de Araujo et al. 2003c; Rolls 2004c; Small and Prescott 2005; Small et al. 2004a). Conversely, swallowing the stimulus resulted in stronger activation in primary chemosensory cortices located in the anterior insula and piriform cortex, consistent with the notion that swallowing is an essential part in the formation of the flavour percept (Burdach and Doty 1987; Burdach et al. 1984).

Overall, both methods of stimulus delivery have certain advantages.

Requesting volunteers to swallow the flavoured stimulus is a more natural way of perceiving flavour and increases the BOLD signal in primary chemosensory cortices. On the other hand, asking participants to hold the stimulus in their mouth while the BOLD signal is acquired results in a greater signal from the OFC which is likely due to less head motion. An additional factor influencing OFC activation may be that the stimulation duration was longer (9s) during the ‘hold’ condition compared to the ‘swallow’ condition (3s). Since OFC responses to olfactory stimulation do not habituate (Sobel et al., 2000; Poellinger et al., 2001), this prolonged stimulation may have resulted in a more sustained signal increases in this region which consequently increases the signal-to-noise ratio and hence detectability. At least the potential motion confounds could be overcome by the use of a bite-bar in future experiments, even though the results of the study using the ‘mouthshim’ would suggest that using such a device would lower signal in insula cortex because of swallowing during the baseline periods of the experiments. However, if subjects were to be cued when to swallow, then swallowing could be explicitly modelled as a variable of no interest in the data analysis. With such improvements implemented, the method of swallowing a stimulus immediately after delivery may prove to be the optimal method for presenting flavour stimuli in future experiments.

9.3 MULTISENSORY VISUAL-OLFACTORY INTEGRATION

The main aim of this thesis was to investigate the neural basis of multisensory integration between vision and olfaction. Previous studies to date had been restricted to behavioural paradigms that predominantly investigated the influence of colours on

the perception of food and drinks (Christensen 1985; Clydesdale 1993; King and Duineveld 1998; Kostyla and Clydesdale 1978). These studies suggest that appropriately coloured stimuli (e.g. yellow lemon juice) are perceived as more pleasant and intense and are easier to identify than inappropriately coloured stimuli (e.g. blue lemon juice) (DuBose et al. 1980; Morrot et al. 2001; Zellner et al. 1991; Zellner and Kautz 1990). The majority of studies reporting such colour induced behavioural enhancements utilised odour-colour combinations derived from natural associations of fruits and their characteristic colour (e.g. strawberry and red), which suggests that these are acquired through a mechanism of associative learning (Clydesdale 1993; Gottfried and Dolan 2003; Rolls 2006; Rolls and Baylis 1994; Rolls et al. 1996a). Interestingly, two studies also reported that some fragrances are consistently matched to certain colours (Gilbert et al. 1996; Schifferstein and Tanudjaja 2004). Since none of the fragrances are associated with any naturally occurring object, the reason behind such a robust colour association remains to be determined. One explanation is that certain components of these perfumes have existing colour associations which influence the responses of participants (e.g. citrus and yellow/orange). Additionally, perfumes can be broadly divided into different olfactory categories such as Floral, Oriental (spicy), Citrus or Marine. Frequently, fragranced consumer products (i.e. shower gels, soaps, etc.) within a certain category are consistently coloured in the same hue (e.g. products of the Marine odour category are typically coloured blue). It is therefore likely that even for such ‘unnatural’ odours, colour associations are acquired by a mechanism of associative learning, rather than constituting an intrinsic property of these odours.

An initial behavioural study was carried out to investigate existing colour-odour associations and determine if participants could perform such a matching task

when colours are presented on a computer screen (Chapter 6), as would be the case for subsequent fMRI investigations. The odours used for this study were pre-selected on the basis that they had previously been reported as having certain colour associations such as strawberry –red (Gilbert et al. 1996; Zellner et al. 1991). Unsurprisingly, the results confirmed that most odours were consistently matched to a certain colour. However, a few odours did not show the expected colour associations. Notably, the smell of cinnamon which has previously been shown to be associated with the colour red (Gilbert et al. 1996) was associated with brown/orange in the present study. This finding may be explained by the observation that the American population studied by Gilbert and colleagues may have been frequently exposed to cinnamon flavoured sweets which are predominantly coloured red, whereas the European sample investigated in the present study may have stronger associations with the cinnamon spice which is coloured light brown. Importantly, this finding strongly suggests that colour associations are acquired and may therefore be subject to cross-cultural differences (Ayabe-Kanamura et al. 1998; Chrea et al. 2005).

Overall, this behavioural study confirmed the existence of consistent colour-odour associations and also informed the selection of 4 different odours with strong colour associations for a subsequent fMRI investigation described below.

To investigate the neural mechanisms involved in colour-odour integration, a fMRI experiment was carried out with 4 different odours (lemon, strawberry, spearmint and caramel) that were each strongly associated with certain colours (yellow, red, turquoise and brown). During this experiment, participants were exposed to the odours and colours either in isolation, or in both congruent (e.g. spearmint – turquoise) and incongruent (e.g. spearmint – brown) combinations and were required to assess the degree of congruency for the bimodal stimuli on a rating scale.

The primary finding of this study was that neural activation in the left OFC and insula cortex increased with the perceived congruency between colours and odours. This observation is consistent with electrophysiological studies in primates that reported bi-modal neurones in OFC which respond to both the sight and smell of food items (Rolls and Baylis 1994; Rolls et al. 1996a) and previous fMRI investigations on odour-picture congruency (Gottfried and Dolan 2003). Since the pleasantness of olfactory stimuli is encoded in OFC as well (Anderson et al. 2003; de Araujo et al. 2003c; Rolls 2006; Royet et al. 2001), this increase in neural activity in OFC may be associated with the increase in perceived pleasantness for congruently coloured flavour solutions (Zellner et al. 1991). Additionally, the left insula cortex displayed responses similar to those observed in the OFC. This region receives multimodal inputs from the chemical senses and are involved in flavour processing (Cerf-Ducastel and Murphy 2001; de Araujo et al. 2003c; Savic et al. 2002; Weismann et al. 2001). To the extent that flavours are primarily determined by their olfactory components (Murphy et al. 1977), this finding suggests that insula olfactory and flavour processing areas also receive visual inputs which in turn may influence the perceived aromas of food and drinks as previously shown in behavioural studies (Clydesdale 1993; King and Duineveld 1998; Kostyla and Clydesdale 1978).

A further analysis of the data was specifically aimed at the detection of the two canonical multisensory mechanisms of superadditivity and response depression (Calvert et al. 2001; Calvert et al. 2004; Macaluso and Driver 2005). The results of this analysis demonstrated that responses in OFC to congruent colour-smell combinations can exceed the sum of the responses obtained when colours and odours are presented in isolation. This finding is in agreement with previous studies that reported superadditive responses in OFC for congruent visual-olfactory (Gottfried and

Dolan 2003) and olfactory-gustatory (de Araujo et al. 2003c; Gottfried and Dolan 2003; Small and Prescott 2005; Small et al. 2004a) stimulus combinations.

Conversely, incongruent colour-odour stimuli did not suppress the responses below those observable after unimodal stimulus presentation. A potential reason for this may be that the mechanism of response depression is primarily used in the processing of spatial information by our nervous system (Calvert 2001; Macaluso and Driver 2005). Because visual-olfactory congruency seems to primarily influence intensity and pleasantness perception, rather than the spatial characteristics of the olfactory percept, it appears that the spatial locations of odours are not represented well in the human brain.

A further finding from this study was that the putative sites for multisensory integration were lateralised to the left hemisphere, a trend that was also present in the only other fMRI study concerned with olfactory-visual integration (Gottfried and Dolan 2003). To rule out the possibility that this laterality effect was caused by colours alone rather than more complex visual depictions of objects, a second fMRI experiment was conducted (Chapter 8). In this study, seven different odours (orange, apple, banana, strawberry, cheese and spearmint and pine) were shown either together with congruent or incongruent pictures while subjects were required to assess the congruency between them.

The results of this study again showed a network of brain areas that responded increasingly stronger to more congruent bimodal stimuli. Importantly, this congruency dependent network included the OFC, the piriform cortex and adjacent amygdala and hippocampus and was lateralised to the left hemisphere. The only bilateral congruency depended activation was observed in the parahippocampal gyrus. Taken together, this finding suggests that the multisensory integration between olfaction and

vision predominantly occurs in the left cerebral hemisphere. Such a left lateralised network may at least partly explain the ‘tip-of-the-nose’ phenomenon, where people cannot name an odour even though they report that they definitely know the odour (Desor and Beauchamp 1974; Jonsson and Olsson 2003; Lawless and Engen 1977). Since semantic associations of odours appear to be primarily encoded in the left hemisphere but odours in the absence of visual cues are primarily represented in the right as suggested by others (Royet et al. 2001; Royet et al. 1999; Zatorre et al. 1992) than the absence of visual cues may lead to predominantly right hemispheric odour processing which only has diminished access to the semantic information.

A further novel finding to emerge from this study was the observation that visual inputs could modulate brain responses in primary olfactory cortex. Since behavioural studies suggest that visual-olfactory congruency may influence intensity judgements (Zellner and Kautz 1990) and odour intensity is encoded in piriform cortex (Anderson et al. 2003; Gottfried et al. 2006). It is feasible to speculate that interactions in piriform cortex are responsible for the enhancements observed behaviourally. Because of the low temporal resolution of fMRI it is not possible to determine whether these interactions occur with inputs originating from the olfactory bulb or are a result of back-projections from the OFC, a potential research question for future MEG experiments.

To conclude, both behavioural and fMRI evidence presented in this thesis suggest that visual cues can influence olfactory perception and that the primary location for these interactions to take place is located in the left orbitofrontal cortex.

9.4 FUTURE DIRECTIONS

Two interesting distinctions between olfactory–visual interactions and audio–visual or visual–tactile ones have emerged from the work presented in this thesis. Firstly, it appears that multisensory interactions involving olfaction may be unidirectional in that, unlike the reciprocal influences for other modalities (see Calvert et al. 2004 for an extensive overview), only effects of vision on olfactory perception have been reported in both psychophysical and neuroimaging literature. Whether odours can influence visual perception remains to be determined. These results fit nicely into the growing body of empirical literature demonstrating that in many circumstances, visual cues will dominate over those presented to other modalities - an effect referred to as visual dominance (Driver and Spence 2000; Posner et al. 1976).

An alternative explanation for the failure to observe odour-induced modulation of visual cortex could be the poor spatial resolution of the human olfactory system, which does not enable us to accurately detect the source of an odour solely on the basis of olfactory information. Instead, humans heavily rely on visual information to locate the spatial origin of a smell. This of course means that it is not feasible to vary the spatial congruency of olfactory-visual stimuli in a fashion similar to audio-visual or visual-tactile experiments and therefore a direct comparison of the mechanisms of multisensory integration of different sensory combinations is not possible. An interesting question for future investigations is therefore to define the conditions under which odours are perceived as emanating from an object and not as an ambient smell.

Secondly, the integration of olfactory cues with visual inputs appears to take place primarily in secondary olfactory areas, particularly in the orbitofrontal cortex, even though there is some evidence that primary olfactory cortex may be influenced

by vision. Whether these interactions modulate the corresponding primary (in this case, olfactory) cortex directly or via back-projections from higher order areas as has been claimed in the case of audio-tactile and audio-visual integration (Calvert 2001; Calvert et al. 2004; Kayser et al. 2005; Lutkenhoner et al. 2002; Soto-Faraco et al. 2004) remains to be determined. The question whether modulation in primary sensory cortices is a result of back projections from higher order areas or emerges from direct contact between the sensory modalities via direct connections, correspond to the late and early models of integration respectively (Calvert and Thesen 2004). Therefore, future research could address this issue either by anatomical tracer studies or by neuroimaging methods with high temporal resolution such as MEG.

The experiments described in this thesis have investigated the neural representations of existing visual-olfactory associations. Therefore, an interesting future research question would be to investigate the formation of these associations. In such a potential olfactory association experiment, participants would be repeatedly exposed to a 'novel' odour (i.e. an odour that has no previous association to an object, such as a new fragrance) which is consistently paired with a novel object (e.g. a certain shape of a particular colour). Brain responses to this odour-object combination at the beginning of this experiment (when no association exists between odour and object) could then be compared to those at the end of the experiment (after an association has been formed). For such an experiment, it seems feasible to speculate that the same brain regions involved in the processing of previously encountered combinations (i.e. OFC and piriform cortex) would also be engaged during learning of such novel odour-object combinations and would therefore respond increasingly stronger as the association is established. Furthermore, forming such an association

could also have behavioural consequences, for example an increase in the pleasantness of the odour when shown together with the appropriate shape.

One of the interesting findings of the behavioural colour-odour matching study (Chapter 6) was that the cinnamon odour was matched to the colours orange and brown (the colour of the spice) and not to red (like cinnamon flavoured products) as previously suggested in a similar study conducted in the USA (Gilbert et al. 1996). These observations suggest that colour-odour associations are acquired and subject to cross-cultural differences. Future investigation could more formally investigate such systematic differences and use these results in the design of brain imaging experiments. For example, one would expect enhanced activation in OFC and piriform cortex for the combination cinnamon-brown compared to the cinnamon-red combination when UK subjects are tested and exactly the reverse for USA subjects.

Future studies could also investigate the relative importance that various visual features play in the formation of visual-olfactory associations. Even though the psychological literature suggests that colour is a dominant factor, it would be of interest to investigate if colour-blind people form similar associations based on other visual features, such as brightness and saturation.

Finally, it would be of interest to study smell-colour synaesthetes to investigate if they display 'normal' colour-smell associations or highly idiosyncratic ones that have no relation to existing objects (e.g. perceive the smell of strawberries as blue). The only obstacle for such a study would be that synaesthesias involving the chemical senses are particularly rare, even though some case studies have been published including taste-sound synaesthesia (Beeli et al. 2005) and lexical-gustatory synaesthesia (Simner and Ward 2006).

Investigating under precisely which conditions visual-olfactory associations are learned best and exert the strongest behavioural effects (such as increased pleasantness) could be a valuable aid in the design of novel food and drink items, particularly for those with a diminished sense of smell.

10 References

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