INVESTIGATING THE EFFECTS OF ODOURS ON PERCEPTION USING EEG AND FMRI

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Stephanie Cook

January 2017

Frequent abbreviations

ANOVA	Analysis of variance
BOLD	Blood oxygenation level dependent
dmPFC	Dorsomedial prefrontal cortex
EEG	Electroencephalography
ERP	Event-related potential
EPI	Echo planar imaging
FMRI	Functional magnetic resonance imaging
FWE	Family-wise error
HRF	Haemodynamic response function
LPP	Late-positive potential
MNI	Montreal Neuroimaging Institute
MRI	Magnetic resonance imaging
OFC	Orbitofrontal cortex
RF	Radiofrequency
ROI	Region of interest
SOA	Stimulus onset asynchrony
TE	Time to echo
TR	Time to repeat
vmPFC	Ventromedial prefrontal cortex

Contents

List of Figures

List of Tables

This thesis is submitted in fulfilment of the conditions for a PhD by published papers. In accordance with the University of Liverpool guidelines and regulations the experimental chapters (Chapters 3 to 6) of this thesis will take the form of journal article manuscripts, which have either been published during the preparation of this thesis, are under review in a peer-reviewed journal, or are being read by co-authors before submission to a peer-reviewed journal. Specific details with regards to journal submission and contribution of authors are given at the beginning of each chapter, as required.

Investigating the effects of odours on perception using EEG and fMRI

Stephanie Cook

Abstract

Olfaction and emotion are tightly linked due to the close anatomical coupling of the two systems in the brain. As a result, odours provide an effective means of manipulating hedonic perceptions of other stimuli. This thesis set out to explore the neural mechanisms underlying such effects.

Using ERP analysis and event-related fMRI, we investigated how pleasant and unpleasant odours affected hedonic evaluations of visual stimuli, and examined whether these effects were dependent on the timing of stimulus presentation, olfactory-visual congruency, and the focus of the rating task. We also explored bidirectional cross-modal effects of visual stimuli on odour pleasantness and intensity perception.

We found that odours consistently modulated hedonic evaluations of faces, objects and flowers, and that these visual stimuli in turn affected odour pleasantness and intensity ratings, and respiratory patterns. Effects of odours on face perception were represented in mid- and late-ERP components. Simultaneous olfactory-visual stimulation and olfactory-visual congruency amplified such effects, particularly in the context of an unpleasant odour. fMRI data showed that activity in regions known as part of the brain's valuation system was related to subjective hedonic ratings, and was boosted by a pleasant odour context.

This thesis concludes that odours exert robust effects on hedonic evaluations. Moreover, visual stimuli in turn influence odour perception. The resulting changes in neural activations and respiratory patterns are likely the result of an evolutionary adaptive mechanism responding to ecologically relevant cross-modal information. Effects of odours on hedonic evaluations are represented in mid- and late-ERP components and by activity in the brain's valuation system.

Declaration

No part of this work has been submitted in support of any other application for degree or qualification at this or any other University or institute of learning.

Acknowledgements

First and foremost I would like to thank my primary supervisor, Dr Andrej Stancak, whose guidance, training and support was invaluable and surpassed all of my expectations. Without his expertise, attention to detail, and passion for the subject none of this would have been possible. Many thanks also to Dr Timo Giesbrecht and Professor Matt Field for their insight, supervision and advice.

I would also like to thank the fellow members of my research group, with a special thanks to Nick Fallon for providing further training and support, and being a great mentor. Thank you to John Tyson-Carr, Hazel Wright, Katerina Kokmotou, Vicente Soto, Moon Wilton and Julia Jones who were all so generous with their help. It was a pleasure working with you all and I hope that we may stay in touch and collaborate in the future!

I am deeply grateful to the ESRC and Unilever for funding this project, and would also like to thank all of the study participants for their time and patience during experiments.

On a personal note I would like to thank my Mum and Dad, you guys are my rock and my inspiration to work hard every day. Thanks to my family, friends and loved ones who were there for me when I needed support and encouraged me to keep going when things got tough. I couldn't have done it without you.

Chapter 1

General introduction

1.1 Olfactory perception

Despite the relative neglect of olfactory research in comparison to visual and auditory modalities, it is widely recognised that the olfactory system plays a significant role in human memory, emotion and cognition (Engen, 1973; Engen & Ross, 1973; Richardson & Zucco, 1989). Odour molecules are volatile chemicals detected by olfactory sensory neurons (OSNs). Dendrites of OSNs extend into the olfactory epithelium, which encompasses thin cilia that protrude into the nasal mucosa; the mucus that lines the nasal cavity (Buck & Bargmann, 2000; Mackay-Sim & Royet, 2006). The cilia contain odour receptors that recognise and bind to odorants. An odorant binding to its receptor induces intracellular signalling events that depolarise the OSN (Buck & Bargmann, 2000). Axons of OSNs pass through a perforated region in the skull above the nasal cavity, known as the cribriform plate, where they synapse on glomeruli with mitral and tufted cell relay neurons in the ipsilateral olfactory bulb (Buck & Bargmann, 2000; Firestein, 2001). These relay neuron axons then project to olfactory cortex, where they terminate on dendrites of pyramidal neurons whose axons project to other brain areas (Buck & Bargmann, 2000) (see Figure 1.1).

1.2 Olfactory cortex

Early investigations of projections from the olfactory bulb were conducted using animals, and were the first to suggest close connections between the olfactory and limbic systems in the brain (Kay & Freeman, 1998; Rolls & Baylis, 1994; Tanabe, Yarita, Iino, Ooshima, & Takagi, 1975). Electrophysiological studies observed gamma band activity in the olfactory bulb of the hedgehog (Adrian, 1950), and in the olfactory bulb and pre-piriform cortex of other mammals (Bressler &

Freeman, 1980). Gamma band frequencies varied with olfactory bulb size (Bressler & Freeman, 1980), and coherence and correlation analysis indicated activity being passed from the periphery inward within and between olfactory bulb, pre-piriform cortex and entorhinal cortex (Boeijinga & Da Silva, 1988, 1989; Bressler, 1987a, 1987b).

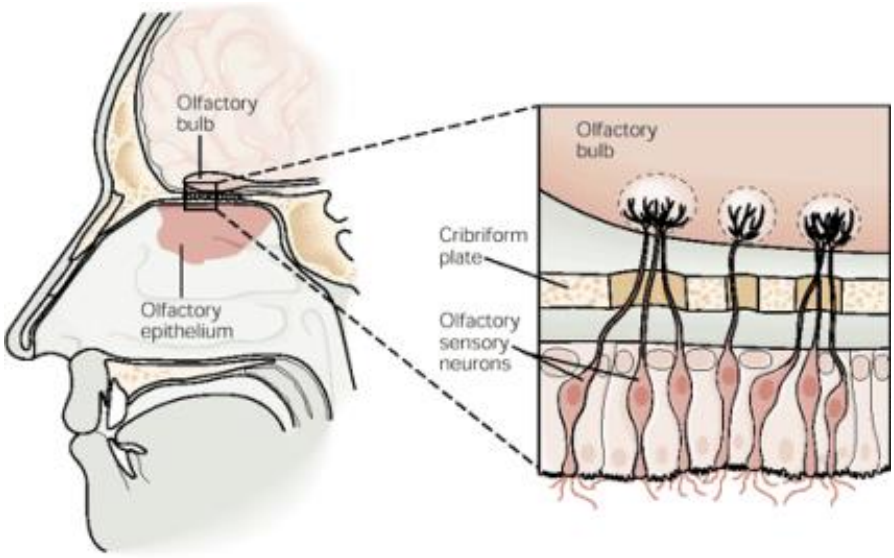

Figure 1.1: A basic schematic of the olfactory system, representing the dendrites of olfactory sensory neurons extending into the olfactory epithelium in the nasal cavity, whilst their axons protrude through the cribriform plate and synapse in the olfactory bulb. From Buck and Bargmann (2000).

In line with the animal data, olfactory cortex in humans is very closely related with subcortical, limbic regions of the brain known for their involvement in emotion and memory (LaBar & Cabeza, 2006; LeDoux, 1993; Phelps, 2004). The primary olfactory cortex encompasses regions receiving direct projections from the olfactory bulb, including the anterior olfactory nucleus, anterior and posterior piriform cortex and amygdala, the olfactory tubercle and entorhinal cortex. The piriform cortex is the largest of these areas, and is considered the major part of the primary olfactory cortex (Buck & Bargmann, 2000; Carmichael, Clugnet, & Price, 1994; Price, 1985). Olfactory information is transmitted from the primary olfactory cortex in two pathways; one pathway relays sensory information directly to other brain structures, bypassing the thalamus (a process that is unique to olfaction) (Ongur & Price, 2000; Shepherd, 2005), whilst the other pathway relays sensory information indirectly

through the thalamus, like other sensory modalities (Buck & Bargmann, 2000). These higher order projections converge on secondary olfactory regions in frontal and orbital areas of the neocortex, which include the orbitofrontal cortex (OFC), agranular insula, amygdala subnuclei, hypothalamus, and hippocampus (Buck & Bargmann, 2000). Secondary olfactory regions are known to be important for odour discrimination, and emotional and physiological responses to odours (Buck & Bargmann, 2000). In a recent meta-analysis, Seubert, Freiherr, Djordjevic, and Lundstrom (2012) showed that areas consistently named as part of primary and secondary olfactory cortex are reliably so (see Figure 1.2). The role of the primary and secondary olfactory cortices will now be discussed in further detail, with particular emphasis on the piriform cortex, amygdala and OFC.

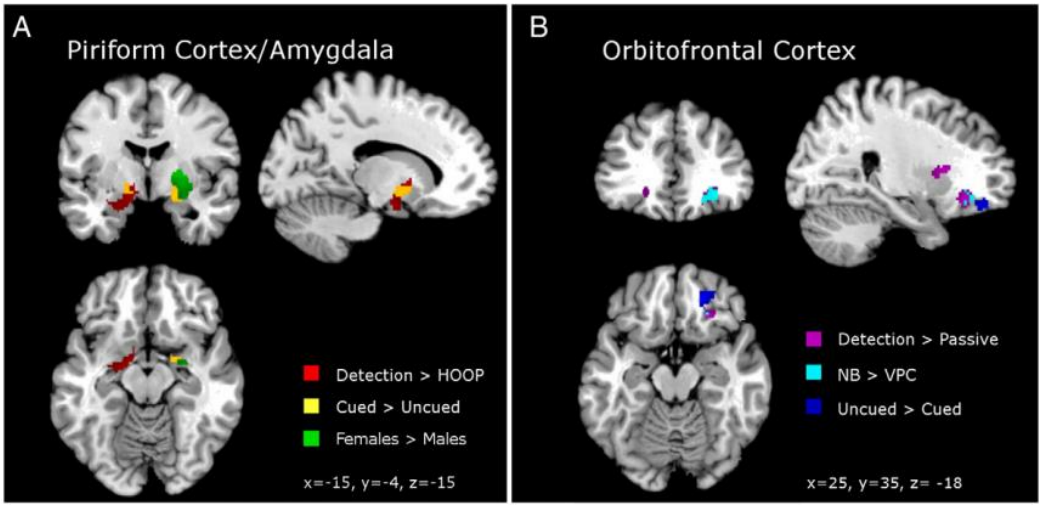

Figure 1.2: Primary and secondary olfactory cortex. From Seubert et al. (2012).

1.2.1 Piriform cortex

As mentioned, piriform cortex is considered the key primary olfactory area and is the major recipient of inputs from the olfactory bulb (Buck & Bargmann, 2000; Gottfried & Zald, 2005). The piriform cortex responds to both smells and sniffing in the absence of an odour (Sobel et al., 1998), and is receptive to hedonic quality as well as sensory perception (Gottfried, Smith, Rugg, & Dolan, 2004; Savic, Gulyas, Larsson, & Roland, 2000). It is thought that the piriform may be involved in

odour recognition and memory, and possibly odour familiarity (Mackay-Sim & Royet, 2006; Royet et al., 1999). Neuroimaging research has encountered inconsistent activation of the piriform during olfactory stimulation (Royet et al., 1999; Sobel et al., 1998; Zald & Pardo, 1997; Zatorre, Jones-Gotman, Evans, & Meyer, 1992), thought to be due to conventional fMRI sequences resulting in signal loss in olfactory specific areas, and olfactory habituation. However, event-related designs and special imaging protocols have been employed in more recent years in order to avoid such methodological issues (Gottfried, 2006).

1.2.2 Amygdala

The amygdala receives direct projections from the olfactory bulb (Gottfried, 2006) as well as secondary projections from primary olfactory areas (Buck & Bargmann, 2000), and is therefore referred to as both a primary and secondary olfactory region (Gottfried & Zald, 2005). Hence, odour induced activations are observed in the amygdala and its function includes basic perception of odours, but also higher order processes of affect, learning and motivation (Mackay-Sim & Royet, 2006). The amygdala is thought to encode odour valence and/or intensity; however, experimental findings have been mixed. Early research suggested that the amygdala encoded odour valence (Zald & Pardo, 1997), but the findings were criticised due to the confound of odour intensity. Later research instead suggested that the amygdala encoded intensity (Anderson et al., 2003). However, a study controlling for both valence and intensity showed that amygdala responded to intensity for pleasant and unpleasant odours, but not neutral odours, suggesting that the interaction between intensity and valence, and therefore the overall behavioural salience of the odour was reflected by amygdala activation (Winston, Gottfried, Kilner, & Dolan, 2005). More recent research has suggested that the amygdala encodes the complete spectrum of odour valence (Jin, Zelano, Gottfried, & Mohanty, 2015).

Neurons in the olfactory bulb project to specific areas of the amygdala that transmit signals to the hypothalamus, which may be responsible for controlling appetite, reproductive behaviours and memory for odours (Buck & Bargmann, 2000). The amygdala is now known to be involved in associative learning between visual

stimuli and olfactory reinforcers, and encoding predictive reward value (Gottfried, O'Doherty, & Dolan, 2003). Further, amygdala activation has been associated with the evocation of odour memories (Herz, Eliassen, Beland, & Souza, 2004), supported by lesion studies of patients with bilateral amygdala damage (Buchanan, Tranel, & Adolphs, 2003; Markowitsch et al., 1994).

1.2.3 Orbitofrontal cortex (OFC)

Early research implicating the OFC as part of the olfactory cortex came from studies investigating single neuron recordings in monkeys (Critchley & Rolls, 1996; Rolls, Critchley, & Treves, 1996). Electrophysiological and histochemical evidence showed that discrete portions of the monkey OFC receive direct afferent inputs from primary olfactory cortex (Gottfried & Zald, 2005). Data from humans demonstrated substantial cytoarchitectural convergence between the monkey and human OFC, with authors suggesting that the parallel organisation in monkeys and humans would allow experimental data from monkeys to be applied to studies of the human cortex (Ongur, Ferry, & Price, 2003). However, functional neuroimaging studies revealed that the human OFC shows an olfactory responsive region in a location more anterior (or rostral) to that predicted from the monkey data (Gottfried & Zald, 2005). Authors have suggested that this may be due to technical and methodological issues, translocation of cell position, or, most likely, an evolutionary change in the relative importance of anterior vs posterior regions, whilst neural networks remained the same (Gottfried & Zald, 2005). Hence, the reliability of translation from monkey to human data on the OFC is still debated (Gottfried & Zald, 2005).

Gottfried and Zald (2005) compared data from 5 PET and fMRI studies on human olfaction, and found that the localisation of olfactory OFC in humans was highly consistent across studies. The OFC is the major neocortical area receiving direct afferent inputs from all regions in the primary olfactory cortex (apart from the olfactory tubercle) without a thalamic relay, and is known as the secondary olfactory cortex. It is located along the basal surface of the caudal frontal lobes, including the gyrus rectus medially and the agranular insula laterally, which wraps onto the caudal orbital surface (Gottfried, 2006). The topography of the human OFC retains the basic

features of the monkey OFC, however, a deep horizontally running sulcus bisects the middle orbital region between medial and lateral orbital sulci (Gottfried & Zald, 2005). Interestingly, pleasant odours are known to evoke stronger activity in medial OFC, whilst unpleasant odours activate lateral OFC (Gottfried, 2006; Gottfried, O'Doherty, & Dolan, 2002; Rolls, Kringelbach, & de Araujo, 2003; Seubert et al., 2012).

Caudal OFC activation is typically associated with low level olfactory processing, such as passive smelling and odour detection (Gottfried, Deichmann, Winston, & Dolan, 2002; Zald & Pardo, 1997; Zatorre et al., 1992), suggesting that this region represents the initial neocortical projection site from the primary olfactory cortex (Gottfried, 2006). Lesions of the OFC have been associated with impairments in odour identification (Jones-Gotman & Zatorre, 1988), odour quality discrimination (Potter & Butters, 1980), and olfactory memory (Jones-Gotman & Zatorre, 1993). Patients with OFC lesions were also impaired on the University of Pennsylvania Smell Identification Test (UPSIT) (Doty, Shaman, Kimmelman, & Dann, 1984; Gottfried & Zald, 2005). However, such studies showed that detection thresholds were relatively preserved with orbital lesions. Authors have argued that the absence of lesion effects on elementary processing implies that the olfactory projection site in human OFC may not have direct access to representations of odour perception, and may instead receive only highly refined and abstracted sensory inputs in order to process more complex olfactory behaviours (Gottfried & Zald, 2005).

Indeed, the role of the OFC in higher order cognitive operations related to odour processing has been discussed (Gottfried & Zald, 2005). Odour intensity judgements (Zatorre, Jones-Gotman, & Rouby, 2000), familiarity judgements (Royet et al., 2001; Royet et al., 1999), hedonicity judgements (Royet et al., 2001) and quality discrimination (Savic et al., 2000) are all associated with orbitofrontal activity. Studies have shown that the reward value of odours is represented in the OFC, leading to the conclusion that the OFC records affect-related, rather than sensory-related processing of odour stimuli (Gottfried & Zald, 2005; Grabenhorst & Rolls, 2011; Grabenhorst, Rolls, Margot, da Silva, & Velazco, 2007; Rolls, 2004a). In particular, one such study showed greater activations in the OFC when the task was to rate and remember odour pleasantness compared to when the task was to rate odour intensity (Rolls, Grabenhorst, Margot, da Silva, & Velazco, 2008). Moreover,

another study showed that medial OFC tracked the absolute subjective value of pleasant and unpleasant odours, whilst the anterolateral OFC tracked the subjective value of the odours relative to a more or less pleasant odour (Grabenhorst & Rolls, 2009).

Studies have shown that more rostral areas of the OFC are engaged in higher order olfactory computations, associative learning (Gottfried & Dolan, 2004; Gottfried, O'Doherty, et al., 2002; Gottfried et al., 2003) and odour recognition memory (Dade, Zatorre, & Jones-Gotman, 2002; Savic et al., 2000). Moreover, cognitive tasks influenced responses to odours in the OFC (Royet & Plailly, 2004). OFC activations are usually accompanied by regional responses in large areas of the frontal, temporal, parietal and occipital cortex (in absence of primary olfactory cortex), suggesting the involvement of non-olfactory networks in mediating higher level olfactory decision making (Gottfried, 2006). An increasing body of research has implied that the OFC is involved in cross-modal integration, in particular olfactory-visual convergence. One such study showed that bimodal stimulus pairs appear to be processed more rostrally, whereas odours alone were processed more caudally (Gottfried & Dolan, 2003), providing further evidence for the involvement of caudal and rostral OFC in basic and higher-order olfactory processing, respectively.

1.3 Olfaction and emotion

From the research discussed, it is clear that the olfactory system has several unique properties compared to other sensory modalities: the ipsilateral nature of central projections, the absence of thalamic intermediary in one pathway between olfactory cortex and higher-order structures, and an intimate overlap with limbic brain regions (Gottfried, 2006). The latter two of these properties are likely responsible for the overlap between olfaction and emotion. It has been argued that the close links between olfaction, emotion and memory are a logical consequence of the anatomical relationship between the olfactory and limbic systems (Royet, Plailly, Delon-Martin, Kareken, & Segebarth, 2003). For many animals, odours motivate almost every aspect of behaviour: maternal bonding, kinship recognition, food search, mate selection, predatory avoidance and territorial marking (Gottfried, 2006),

and much of the fundamental evidence for the relationship between olfaction and emotion in the brain comes from studies in non-human primates (Rolls, 2004a, 2004b). However, as shown by the recent neuroimaging studies in humans discussed, there is strong neurological evidence to suggest a close anatomical coupling of olfactory and emotional processes in the brain (Gottfried, Deichmann, et al., 2002). We recognise from our own human experience that odours have an extraordinary ability to trigger memories, and can modulate emotional reactions and changes in mood with little cognitive involvement (Richardson & Zucco, 1989).

Olfactory research has provided increasing evidence that odours are automatically affective stimuli. We involuntarily categorise odours by their pleasantness, and so emotion and hedonic judgement are primary facets of olfaction (Bensafi, Rouby, Farget, Bertrand, et al., 2002; Herz & Engen, 1996). It has been suggested that hedonic factors are of such considerable importance in odour categorisation due to the relatively small vocabulary assigned to describing odours; odours are typically described as part of the complex emotional experiences in which they are encountered, rather than through the use of specific words or phrases (Richardson & Zucco, 1989). Indeed, early olfactory research suggested that neutral odours may acquire values through pairing with emotionally significant events (M. D. Kirk-Smith, Van Toller, & Dodd, 1983). Thus, odours are inherently pleasant or unpleasant, and very tightly linked with emotions.

It is well known that the inherent pleasantness or unpleasantness of scents can produce approach/avoidance behaviours (Levine & McBurney, 1986; Spangenberg, Crowley, & Henderson, 1996; Takagi, 1989). In everyday life, odours serve as warning signals for threats in our environment (Croy, Drechsler, Hamilton, Hummel, & Olausson, 2016). For negative odours in particular, this warning function is characterised by withdrawal reflexes and disgust (Stevenson, 2010). For example, Miltner, Matjak, Braun, Diekmann, and Brody (1994) showed that an unpleasant odour enhanced startle reflex amplitude relative to neutral air stimulation, whereas a pleasant odour reduced the startle reflex. Additionally, Bensafi, Rouby, Farget, Bertrand, et al. (2002) showed that unpleasant odours provoked heart rate acceleration during a smelling task. More recently, a study by Boesveldt, Frasnelli, Gordon, and Lundstrom (2010) demonstrated that an unpleasant food odour (fish) was detected faster and more accurately than odours belonging to other categories (e.g. rose). The authors concluded that the olfactory system reacts more efficiently to

ecologically relevant stimuli that signal danger. Moreover, pleasant odours are known to produce approach behaviours (e.g. looking time) and improve mood (Knasko, 1995). Hence, odours produce both physiological and psychological approach/avoidance reactions in order to provide an ecological advantage.

1.4 Cross-modal effects of odours

The integration of the olfactory system with other sensory modalities has become an important theme in recent olfactory research, with authors suggesting that the integration of olfactory, visual and auditory stimuli in normal perception is common (Gottfried & Dolan, 2003; Platek, Thomson, & Gallup, 2004; Rolls & Baylis, 1994). Multiple senses work together to combine information to enhance the salience, and reveal more about the nature of meaningful external events, creating unitary perceptual experiences (Stein & Stanford, 2008). Single-neuron recordings have identified specific multisensory neurons in the brain that respond to stimuli from more than a single sense. In basic physiology, multisensory integration is defined as a significant difference between the number of impulses evoked by a cross-modal combination of stimuli and the number evoked by such stimuli individually (Meredith & Stein, 1983). The result of multisensory integration is therefore an enhancement or depression of a neuron's response, and the magnitude of multisensory integration is a measure of the relative physiological salience of an event (Stanford & Stein, 2007). Although much research into multisensory integration has focused on visual-auditory interactions, other such studies have revealed multisensory neurons responding to olfactory, gustatory and visual stimuli, namely in the orbitofrontal cortex (Rolls & Baylis, 1994).

In addition to single-neuron recordings, neurophysiological and functional imaging studies have identified many multisensory cortical regions in humans and non-human primates (Stein & Stanford, 2008). Some of this multisensory research has focussed on olfactory-taste interactions, and has suggested that flavour perception is dependent upon such (Small et al., 2004). In particular, one study showed that whilst umami taste presented alone was not pleasant, the resulting flavour when it was paired with a savoury odour was rated as much more pleasant. Moreover, this taste-odour combination produced much greater activation of the

medial orbitofrontal cortex and pregenual cortex than the sum of the activations by the taste and olfactory components presented separately (McCabe & Rolls, 2007).

As evidenced, odours have such a powerful influence on human behaviour that they automatically attract or repel us as a function of their intrinsic affective qualities (L. J. Ball, Shoker, & Miles, 2010). Because of the strong links between the olfactory and emotional systems in the brain and the resulting capacity of odours to evoke direct emotional reactions with minimal involvement of cognitive activity (Ehrlichman & Halpern, 1988), odours provide an advantageous way to manipulate emotional processes and perceptions of other stimuli. Thus, odours are often used to induce affective states in olfactory research (Bensafi, Rouby, Farget, Bertrand, et al., 2002). Indeed, early research studies showed that pleasant odours significantly improved scores on tension and depression measures (Schiffman, Sattely-Miller, Suggs, & Graham, 1995; Schiffman, Suggs, & Sattely-Miller, 1995), and that unpleasant odours had negative effects on mood, emotional ratings of an environment and the amount of time spent there (Rotton, Barry, Frey, & Soler, 1978). One such study by Millot and Brand (2001) showed that voice pitch was modulated by ambient odours, where pleasant odour conditions produced higher voice pitch than unpleasant odour conditions during a reading task. The authors hypothesised a functional convergence of encoding emotion and hedonic perception of odours. Another very recent study showed that odours modulated touch processing (Croy et al., 2016). Thus, the multisensory integration of odours with other sensory stimuli is common.

1.4.1 Odour priming

A number of studies have demonstrated that odours influence perceptions of other stimuli, in particular visual stimuli. These types of effects are referred to as ‘odour priming’ throughout the present thesis. Odour priming effects can refer to the manipulation of preferences for neutral, unrelated stimuli. Such effects are often investigated in ‘evaluative conditioning’ paradigms, where a change in the liking of a stimulus (conditioned stimulus; CS) comes as a result of pairing that stimulus with other positive and negative stimuli (unconditioned stimulus, US) (Hofmann, De Houwer, Perugini, Baeyens, & Crombez, 2010). Alternatively, odour primes can

influence responses to a congruent or incongruent visual stimulus target in 'evaluative priming' type scenarios (Herring, Taylor, White, & Crites Jr, 2011)

1.4.1.1 Effects of odours on face perception

In general, pleasant odours improve hedonic ratings of visual stimuli, and unpleasant odours have the opposite effect. van Reekum, vann de Berg, and Frijda (1999) found that evaluations of abstract paintings presented together with odours were shifted in the direction of the odour valence. In other words, emotionally neutral paintings paired with liked odours were rated more favourably than those presented with disliked odours. The authors concluded that affective odours having no logical connection to pictures were able to induce odour evaluative conditioning. In a much more recent study, adding unpleasant odours to pleasant or neutral images reduced hedonic ratings of the images (Banks, Ng, & Jones-Gotman, 2012).

Thus, pleasant and unpleasant odours are able to modulate evaluations of objects that would otherwise be neutral. Given the vast amount of products on the market with the aim of increasing or decreasing odour according to situational needs, intuition would assume that pleasant and unpleasant odours also affect judgements of other people. Indeed, marketing schemes promote the notion that odours have a direct role in the success or failure of social relationships (Todrank, Byrnes, Wrzesniewski, & Rozin, 1995). Both human face processing and odour processing almost always involve some aspect of emotion (Walla, 2008). Odours increase looking time for faces, and these effects are present from a very early age (Durand, Baudouin, Lewkowicz, Goubet, & Schaal, 2013). Furthermore, research has shown that both pleasant and unpleasant odours improve recognition accuracy for human faces, with pleasant odours having a greater influence (Walla, 2008; Walla, Mayer, Deecke, & Lang, 2005). However, surprisingly little scientific research has sought to back up the assumption that odours influence subjective evaluations of faces. Very early chemosensory research indicated that pheromones were able to modulate evaluations of others. These effects were most prominent in assessments of men by women (Cowley, Johnson, & Brooksbank, 1977). Early studies in applied-psychology showed that pleasant odours enhanced evaluations of job applicants, and resulted in increased negotiation goals in the workplace (Baron, 1983, 1990).

One of the first studies directly assessing the effects of odours on judgements of people in an experimental setting was reported in a book chapter by M. D. B. Kirk-Smith, D. A. (1990). Their results showed that, in the presence of a perfume, both men and women rated photographs of men and women as being significantly 'sexier' and 'softer' as compared with a no-perfume condition. No such effect was observed with a banana essence, which according to the authors was rated equally as pleasant as the perfume. However, it was recognised that prolonged presentation of odours resulted in a change in participant self-reported mood, such that participants rated themselves as feeling sexier after exposure. It was suggested that the effect on mood induced by the odours may have given rise to the behavioural effects reported. Another early study combining odours and photos of people in a conditioning paradigm found similar results. Todrank et al. (1995) paired liked, neutral and disliked odours with photographs of neutral (unfamiliar) people of the opposite sex to participants in a conditioning phase. When the photographs were subsequently presented without odours, participant preference ratings for people in the photographs were shifted according to preference ratings for the odours they were originally presented with.

Later chemosensory research demonstrated effects of body odours on social judgements. Women in videos were rated as more stressed, less trustworthy and less competent when participants were simultaneously exposed to untreated samples of stress sweat (Dalton, Mauté, Jaén, & Wilson, 2013). Effects of subliminally presented odours on judgements of people have also been investigated. In a study by Li, Moallem, Paller, and Gottfried (2007), participants rated the likeability of neutral faces after stimulation with pleasant, neutral or unpleasant odours presented below detection thresholds. Results showed that odour valence significantly shifted likeability ratings from participants who lacked conscious awareness of the odours in a subsequent odour detection task. The magnitude of the odour priming effect decreased as sensitivity for odour detection increased. The authors argued that social preferences are subject to influences from odours that escape awareness, whereas the availability of conscious odour information may disrupt such effects.

Despite this, recent studies have demonstrated that odour priming effects can occur with explicit awareness of odours. Dematte, Osterbauer, and Spence (2007) had female participants judge attractiveness of male faces which were simultaneously presented with clean air or one of four odours (2 pleasant, 2

unpleasant; one of each body relevant or irrelevant). Participants rated male faces as significantly less attractive in the presence of an unpleasant odour as compared to when the faces were presented with a pleasant odour or clean air. There was no difference in attractiveness ratings between the pleasant odour and clean air conditions. Furthermore, results were unaffected by whether the odours were body relevant or not. The authors concluded that unpleasant odours, even those bearing no relevance to body odours, have a cross-modal influence on judgements of attractiveness. In a very recent study, Seubert, Gregory, Chamberland, Dessirier, and Lundström (2014) investigated the modulatory effects of odours linearly increasing in pleasantness on attractiveness and age perception of female faces. Odours that were perceived as more pleasant resulted in higher attractiveness ratings. Moreover, a linear increase in perceived facial attractiveness was predicted by a linear increase in perceived odour pleasantness. There were no effects of odour on age perception. The study concluded that odours modulate affective, but not cognitive evaluations of faces. Taken together, the research discussed provides strong evidence that odours are able to modulate preferences for neutral stimuli and human faces.

Very few neuroimaging studies have sought to investigate the neural mechanisms underlying effects of odours on evaluations of faces. One magnetoencephalography (MEG) study showed that neutral face stimuli paired with aversive odours in a conditioning paradigm were subsequently rated as more negative. Emotional modulations were observed at intervals of 50−80 and 130−190 ms following face onset in frontal and occipito-temporal regions of the brain, respectively (Steinberg et al., 2012). In an electroencephalography (EEG) study, Herrmann, Ziegler, Birbaumer, and Flor (2000) administered a pleasant food odour (vanilla) and an unpleasant food odour (rotten yeast) as appetitive and aversive unconditioned stimuli. Throughout the experimental task, slides showing neutral male faces were presented as conditioned stimuli. Participants rated the valence and arousal of the faces whilst EEG was recorded from 9 electrodes. Heart rate, electromyography (EMG) and skin conduction response (SCR) were also measured. Subjective ratings and SCR revealed successful aversive conditioning of faces with the unpleasant odour. However, the pleasant odour failed to produce appetitive conditioning. Odour conditioning elicited stronger amplitude of the late positive component (LPC), and the N100 component was more pronounced in the presence of the pleasant odour. Other cortical effects failed to reach significance. The authors

concluded that odour conditioning was mainly represented by a change in subjective evaluations rather than physiological responses, and suggested that the presence of conditioning with a lack of significant cortical correlates was due either to extremely localised cortical processing of conditioned olfactory cues not detectable from ERPs, and/or to deep subcortical processing (Herrmann et al., 2000).

In another EEG study, Bensafi, Pierson, et al. (2002) measured changes in event-relate potential (ERP) responses to female faces caused by a pleasant odour prime. They tested whether pleasant odour affected emotional judgements, response times, N400 response or LPC in response to faces. Participants were instructed to make a binary choice whether the neutral female faces, presented either in the presence of a pleasant odour or no odour, were pleasant or not. No behavioural effects of odours on evaluations or response times were observed. However, the late component of ERPs evoked by faces was modulated by the presence of a pleasant odour: The LPC evoked by faces judged as unpleasant was significantly more positive than LPC evoked by faces judged as pleasant in the pleasant odour condition. The authors suggested that this may reflect enhanced alert reaction to unpleasant faces preceded by an incongruous pleasant odour. Hence, EEG studies thus far have provided a very limited understanding of the neural mechanisms underlying the influence of odours on evaluations of faces.

One functional magnetic resonance imaging (fMRI) study paired pleasant and unpleasant odours with neutral faces in a conditioning paradigm (Gottfried, O'Doherty, et al., 2002). No subjective ratings of the faces were recorded; however, data showed evidence of appetitive and aversive olfactory learning in the medial and lateral OFC, respectively. The authors argued for the evidence that odours could induce cross-modal associative learning. Another fMRI experiment investigated the effects of odours on attractiveness ratings of neutral male faces (McGlone, Österbauer, Demattè, & Spence, 2013). Faces presented in an unpleasant odour condition were rated significantly less attractive than the same faces presented in a pleasant odour condition, or in the absence of an odour. Furthermore, faces presented in the pleasant odour condition produced significant activations in the medial and lateral OFC and ventral striatum, areas known to be associated with reward processing and value encoding (Lebreton, Jorge, Michel, Thirion, & Pessiglione, 2009; Rolls, 2000), facial attractiveness (O'Doherty et al., 2003), and positively valenced odours (Anderson et al., 2003; Rolls et al., 2003). Faces presented in the

unpleasant odour condition produced activations in the amygdala and anterior insular cortex, areas known to be involved in the representation of negative affect (Sato, Yoshikawa, Kochiyama, & Matsumura, 2004; Wicker et al., 2003) and facial unattractiveness (O'Doherty et al., 2003). Thus, functional imaging studies have provided some initial understanding of neural mechanisms underlying odour priming of hedonic ratings of faces. However, the data is sparse, and distinct temporal representations of odour priming in the brain remain unexplored.

1.4.1.2 Olfactory-visual congruency in odour priming

Odour priming effects are often investigated with a focus on congruency between valenced odours and visual stimuli with affective significance. In one early study, Bone and Jantrania (1992) found that products paired with a congruent scent (e.g. cleaning products with lemon scent) were evaluated more positively than products paired with incongruent scents (e.g. cleaning products with coconut scent). A further study showed that target words were evaluated faster if preceded by a similarly valenced odour, as compared to affectively incongruent odour-word pairs (D. Hermans, Baeyens, & Eelen, 1998).

Eye-movement studies investigating visual-olfactory congruency effects have yielded similar results. Seigneuric, Durand, Jiang, Baudouin, and Schaal (2010) investigated how the processing of visual objects was altered by the presence of olfactory cues, and found that odour related visual cues (e.g. a picture of an orange) were explored faster and for a shorter time in the presence of a congruent odour (e.g. orange odour). Another showed that participants looked longer and more frequently at a corresponding object in the presence of an odour as compared to an odourless condition (Seo, Roidl, Muller, & Negoias, 2010). A further study investigating olfactory-visual congruency showed that unpleasant images combined with unpleasant odours produced a stronger SCR than unpleasant images combined with pleasant odours (Banks et al., 2012).

Congruency effects in olfactory priming have also been shown to modulate visual ERPs and neural activity in EEG and MEG studies (Castle, Van Toller, & Milligan, 2000; Grigor, 1995; Grigor, Van Toller, Behan, & Richardson, 1999;

Sarfarazi, Cave, Richardson, Behan, & Sedgwick, 1999; Walla & Deecke, 2010). In one early study, Grigor (1995) paired food odours with photographs of foods. EEG data showed that the N400 component of the visual ERP was greater in incongruous situations, e.g. when an apple scent was paired with a picture of a loaf of bread. Grigor et al. (1999) later extended these findings by using non-food odours and photographs of objects which were matched or mismatched with the odour. Although N400 peaks were produced for both matched and mismatched conditions, peaks were significantly more negative for the mismatched condition. In a similar study, pictures of flowers, fruit and objects were presented with no odour, rose odour, jasmine odour or citrus odour. Participants were instructed to categorise the pictures by pressing one of two buttons (e.g. flower or fruit) (Sarfarazi et al., 1999). The findings mimicked those of Grigor and colleagues, showing increased negativity of the N400 when the picture did not match the odour. The authors concluded that this N400 effect serves as a measure of relatedness of a sensory stimulus to a previous or ongoing prime. Castle et al. (2000) presented pleasant and unpleasant household odour primes followed by congruent or incongruent visual stimuli. The N400 was more negative in response to incongruent stimuli when a malodour was used as a prime, but not when a pleasant odour was used. The authors suggested that this highlights the importance of hedonically negative stimuli.

These types of congruency effects extend to odour priming studies using human faces as visual stimuli. Such studies have suggested that odours are able to prime face discrimination. In one study, recognition of disgusted faces was improved by presentation of an olfactory stimulus, irrespective of its emotional valence. There were no such effects for other facial expressions (Seubert et al., 2010). Leppanen and Hietanen (2003) showed that happy faces were recognised faster than disgusted faces in the presence of a pleasant odour. The authors reported that this recognition advantage disappeared in the unpleasant odour condition due to the slow recognition of incongruent, happy faces. A recent study showed that the minimum amount of visual information required to correctly perceive an expression was lowered when the odour context was emotionally congruent (Leleu, Demily, et al., 2015).

Furthermore, odours in the form of low-intensity chemosensory signals have been shown to modulate the processing of emotionally-valenced faces. Pause, Ohrt, Prehn, and Ferstl (2004) presented chemosensory anxiety odour or chemosensory control odour before and during sub-threshold presentation of happy, fearful and

neutral faces (11 ms) followed by neutral targets (47 ms). In the control condition, subjects judged neutral targets as significantly more positive when they were primed by happy faces compared to fearful faces. In the anxiety odour condition, the priming effect of happy faces was diminished in females. However, there was no effect of anxiety odour on negative priming effects yielded by the fearful faces. In a similar study also recording EEG, Adolph, Meister, and Pause (2013) presented participants with a chemosensory anxiety odour (sweat taken from subjects before an examination) or a chemosensory control odour (sweat taken from subjects in during a sport activity) during the viewing of anxious facial expressions. Both chemosensory signals modulated the processing of fearful faces. EEG data showed that N170 amplitudes were larger for facial expressions presented in both chemosensory contexts as compared to facial expressions with no chemosensory context. Another study observed that stress sweat enhanced the late LPP in responses to neutral and ambiguous faces (Rubin, Botanov, Hajcak, & Mujica-Parodi, 2012). Further research has suggested that non-chemosensory odour contexts can modulate cortical responses to faces. Leleu, Godard, et al. (2015) found that an aversive odour modulated the P200 by amplifying the difference in response to neutral versus happy and disgusted facial expressions. However, no subjective behavioural responses were gathered. Hence, cross-modal effects of odours on visual perception are often dependent on congruency, and neural mechanisms underlying such effects may be reflected in EEG activity.

1.4.2. Cross-modal effects of other stimuli on odour perception

In the same way that odours can influence perceptions of other stimuli, the opposite is true in that odour perception is extremely malleable, and susceptible to top-down influences. An increasing body of research has demonstrated the role of cross-modal integration in odour perception, and suggests that congruency is also of importance in these effects. For example, Seo and Hummel (2011) presented participants with congruent, incongruent or neutral sounds before and during odour presentation. Odours were rated as more pleasant when paired with a congruent sound. In the second part of their study, participants received pleasant or unpleasant sounds before and during the presentation of pleasant or unpleasant odours. Odour

pleasantness for both pleasant and unpleasant odours was amplified by pleasant sounds. Hedonic ratings of the auditory cues also correlated with odour pleasantness ratings. The authors concluded that auditory cues are able to modulate odour pleasantness.

Other studies have investigated the cross-modal effects of visual, semantic and olfactory stimuli on odour perception. Olofsson, Bowman, Khatibi, and Gottfried (2012) had participants perform an identification task where they indicated whether an odour matched the previously presented word label. Responses were quicker for odours preceded by semantically matching words. In another study, participants made speeded odour discrimination responses for fruit odours while viewing congruent or incongruent colour patches, black and white outline drawings, or a combination of both. Discrimination accuracy was diminished by incongruent shape/odour pairing (Dematte, Sanabria, & Spence, 2009). Similarly, Gottfried and Dolan (2003) provided participants with an olfactory detection task, where odours and pictures were delivered separately or together. Results showed perceptual olfactory facilitation for semantically congruent odour-picture pairs. This behavioural advantage was also associated with neural activity in the anterior hippocampus and rostromedial OFC. Authors have argued for the automaticity of high level visual-olfactory cross-modal interactions (Dematte et al., 2009; Gottfried & Dolan, 2003).

Visual information has been shown to affect odour pleasantness perception as well as odour discrimination. Seo, Arshamian, et al. (2010) found that congruent symbol odour pairs increased perceived pleasantness and intensity of a pleasant odour, and increased the unpleasantness of an unpleasant odour. Furthermore, the congruent symbols produced significantly higher amplitudes and shorter latencies in the N1 peak of olfactory ERPs compared to incongruent symbols. In another such study, a neutral suprathreshold odour was rated as less pleasant and more intense following unpleasant picture presentation, whilst viewing positive images increased reported odour pleasantness (Pollatos et al., 2007). More specifically, de Araujo, Rolls, Velazco, Margot, and Cayeux (2005) investigated how semantic information conveying different valences modulated pleasantness perception of the same odour in an fMRI study. A test odour (isovaleric acid) was presented with one of two visual labels: “cheddar cheese” or “body odour”. The odour was rated significantly more unpleasant when labelled body odour than when labelled cheddar cheese. Differences

in cortical activations modulated by the odour label were observed in the OFC and anterior cingulate cortex (ACC), and correlated with odour pleasantness ratings. Cross-modal influences on odour perception are therefore evident. However, such effects have not yet been investigated using emotional faces as a top-down influence on odour perception. As discussed, both odours and faces are potent triggers of emotion. The literature has not yet addressed potential bidirectional cross-modal effects of odours and faces on odour and face processing.

1.5.1 Integration of olfactory and visual stimuli in the OFC

As discussed, the OFC is among the most consistently activated structures in olfactory imaging experiments (Sobel et al., 2000; Zald & Pardo, 1997; Zatorre et al., 2000), is commonly referred to as secondary olfactory cortex (Carmichael et al., 1994; Price, 1985; Seubert et al., 2012), and encodes the reward value of odours (Gottfried & Zald, 2005; Grabenhorst & Rolls, 2011; Grabenhorst, Rolls, & Parris, 2008; Rolls, 2004a). The OFC is also thought to be involved in the convergence of visual and olfactory information (Gottfried & Dolan, 2003). In non-human primates, individual OFC neurons responding to combined visual and olfactory stimulation have been identified, resulting in the argument for olfactory and visual convergence in the primate OFC (Rolls & Baylis, 1994). More recent studies have observed olfactory-visual interactions in the OFC in an associative conditioning task (Gottfried, O'Doherty, et al., 2002), and in the facilitation of odour perception induced by olfactory-visual congruency (Gottfried & Dolan, 2003). Given the role of the OFC in encoding value of various stimuli, it has been postulated that the OFC may be involved in the transfer of affective value between olfactory and visual modalities (Gottfried, O'Doherty, et al., 2002). Hence, the OFC is likely involved in cross-modal interactions between odours and visual stimuli that influence affective value, and may also depend on olfactory-visual congruency.

1.5.2 The OFC and the brain's valuation system

The OFC is well established in the processing of reward and affective value of fundamentally different affective stimuli, including taste, touch, texture and facial

expression (Rolls & Grabenhorst, 2008). Studies have also identified the role of other areas of the frontal cortex, including the ventromedial prefrontal cortex (vmPFC), in representing subjective pleasantness of different types of rewards (Grabenhorst, D'Souza, Parris, Rolls, & Passingham, 2010). In a recent review paper, Grabenhorst and Rolls (2011) discuss the role of both the OFC and the vmPFC in the computation of expected value, reward outcome and experienced pleasure for different stimuli on a common value scale. One recent study suggested that the OFC encodes subjective value in animals, whilst vmPFC encodes subjective value in humans (Abitbol et al., 2015). A meta-analysis of studies investigating brain representation of subjective value in humans concluded that regions encoding subjective value included vmPFC, dorsomedial prefrontal cortex (dmPFC), striatum, thalamus, and anterior insula (Bartra, McGuire, & Kable, 2013). It has been proposed that such regions form a brain valuation system that encodes preferences and values of different types of objects on a common scale (Bartra et al., 2013; Lebreton et al., 2009).

Recent studies have shown that value-based evaluative processes and related brain structures are activated automatically upon viewing an object; regardless of whether or not the task is to explicitly report the subjective judgement (Kühn & Gallinat, 2012; Lebreton et al., 2009; Levy, Lazzaro, Rutledge, & Glimcher, 2011). Moreover, activations in OFC and vmPFC correlate with subjective emotional experience and pleasantness ratings of affective stimuli or rewards (Grabenhorst et al., 2010; Rolls & Grabenhorst, 2008; Rolls, Grabenhorst, & Parris, 2010). The OFC is clearly involved in the integration of visual and olfactory information. The OFC and other areas of frontal cortex, including vmPFC and dmPFC, are integral for reward processing. However, at present it is not clear how the brain encodes the value of different types of visual stimuli in the presence of odour.

1.6 Interim summary

In light of the research discussed here, it is evident that odours have profound links with emotion and therefore exert potent cross-modal effects on stimulus perception. Such cross-modal influences can be observed with neutral stimuli, or alternatively, stimuli that are affectively congruent or incongruent. In the same way that odours influence visual stimulus perception, visual stimuli are able to influence

odour perception, where congruency also plays a role. Given the inherently affective properties of both odours and faces and their relative social importance, they make appropriate stimuli for investigating olfactory-visual interactions. The OFC and other areas of frontal cortex are involved in encoding the affective value of various stimuli, including odours.

1.7 Research problems

Although it is clear that odours can influence evaluations of visual stimuli, including human faces, the neural mechanisms underlying such effects are poorly understood due to a lack of neuroimaging studies. The majority of studies investigating odour priming effects presented odours and faces simultaneously, which is a potential source of confound in subjective ratings. Moreover, little is known about whether there are differences, either behaviourally or in EEG data, in such odour priming with a temporal lag between odour and face presentation versus simultaneous odour-face presentation. Such a question has a wider relevance for the general literature on evaluative priming.

The effects of congruent and incongruent pairings of odours and emotional face stimuli on face processing have not yet been investigated using EEG. Moreover, it is not known whether such combinations can exert bidirectional cross-modal effects on subjective evaluations of both faces and odours. It is not clear how the brain encodes the value of different types of visual stimuli in the presence of odour, and whether olfactory-visual congruency has an effect on activity in the brain's valuation system and related subjective ratings.

1.8 Thesis chapters

Chapter 2 describes the olfactory stimulation equipment and neuroimaging data collection and analysis methods used in the following experimental chapters, with a particular emphasis on the use of statistical parametric mapping (SPM) as a robust and novel method of EEG analysis.

Chapter 3 discusses an EEG study investigating the effects of pleasant and unpleasant odours on evaluations of happy and disgusted faces, using a one second temporal lag between odour offset and face presentation in order to observe true odour priming effects that carried over to face processing when the odour was no longer present. The study described in Chapter 4 was an EEG study observing such effects both with and without this temporal lag. These studies were carried out to elucidate the time course of neural mechanisms underlying effects of odours on evaluations of faces, and to investigate how stimulus onset asynchrony (SOA) influences such odour priming effects.

Chapter 5 investigated the effects of congruent and incongruent pairings of pleasant and unpleasant odours and happy and disgusted faces on both face and odour perception, using EEG. This study was designed to further explore the nature of olfactory-visual congruency in the brain, and to observe bidirectional priming effects of odours and emotional face stimuli. All three EEG studies employed a novel, exploratory approach to ERP analysis, using SPM.

Chapter 6 reports an event-related fMRI study, investigating the effects of a pleasant odour on value-based versus perceptual decision making about congruent and incongruent visual stimuli. The purpose of this study was to further investigate olfactory-visual congruency in the brain with the advantage of the superior spatial resolution of fMRI, and to explore how such congruency affected value-based judgements, with a particular focus on observing activity in the brain's valuation system.

Throughout the experimental chapters, pleasantness ratings are used to measure subjective valuation of visual stimuli; which include faces, objects and flowers. Pleasantness ratings are a common measure of subjective value (Kühn & Gallinat, 2012) and were employed across experiments in order to maintain consistency. The term 'subjective value' is therefore used throughout the present thesis to refer to value measured by such ratings, rather than value as derived from choices.

Chapter 7 provides a general discussion where results of the experimental chapters are summarised with theoretical implications, limitations are described, and some suggestions for future research are proposed.

1.9 Hypotheses

- Pleasant and unpleasant odours will influence evaluations of neutral and affectively congruent and incongruent visual stimuli.
- Odour priming effects will be represented in the ERP response to faces.
- Effects of odours on evaluations of faces will differ as a function of odour-face stimulus onset asynchrony (SOA).
- Emotional faces will influence ratings of odour pleasantness and intensity.
- Congruent olfactory-visual pairings will modulate activity in the OFC, and such activity will fluctuate depending on value-based versus perceptual focus.

Chapter 2

General Methods

2.1 Olfactometer

Throughout all experiments described in the following chapters, a custom-built, computer controlled, eight-channel olfactometer (Dancer Design, Wirral, UK) was used to deliver olfactory stimuli. The channels were made from fluorinated ethylene propylene (FEP) tubing, which connects glass bottles containing odour mixtures to the participant head piece. The head piece was mounted onto participants using a flexible plastic ring, adjusted such that it sat comfortably on the shoulders, and so that two narrow-diameter FEP tubes were placed approximately one cm below the nostrils. These tubes directed the airflow birhinally. One glass odour bottle contained odourless propylene glycol alone, pumped through continuously to provide a constant flow of 'clean air'. Hence, odours were always embedded within this constant flow of clean air, such that participants would not sense changes in airflow associated with odour presentations (Huart, Legrain, Hummel, Rombaux, & Mouraux, 2012). Propylene glycol was also used to dilute the experimental odours. Airflow was kept constant at 2.5 l/min in EEG experiments, and at 4 l/min in the fMRI set up. This difference in airflow is attributable to the air having to travel a further distance through longer tubes used in the olfactometer set up at the Magnetic Resonance and Image Analysis Research Centre (MARIARC). Very similar configurations have been used successfully in previous experiments (Grabenhorst & Rolls, 2009; McGlone et al., 2013; Rolls et al., 2003).

2.2 Electroencephalography (EEG)

2.2.1 Physiological basis of the EEG signal

Action potentials are discrete spikes in voltage generated in the cell body of neurons, which travel along the axon fibre to excitatory or inhibitory terminals. Neurons in the brain communicate via these action potentials; however, they are brief (10 ms or less) with a very limited potential field (Rowan & Tolunsky, 2003), and are generally not synchronised. As a result, voltage generated from action potentials is not detectable at scalp electrodes (E. J. Speckmann & Elger, 2005). However, when an action potential travels along an axon fibre to an excitatory or inhibitory synapse, a post-synaptic potential occurs, and neurotransmitters bind with the postsynaptic cell membrane. This causes ion channels to open, and a potential develops between intracellular and extracellular space (E. J. Speckmann & Elger, 2005). These potentials, referred to as extracellular field potentials (E.-J. Speckmann, Caspers, & Andersen, 1979), are considerably longer (50–200 ms) and have a greater field (Rowan & Tolunsky, 2003). Thousands of field potentials may occur in a similar location and orientation during a coherent response, due to the macroscopic organisation of dendrites (Fisch, 1999). The summation of such potentials may then be detected and measured as a voltage difference on the scalp using EEG (Lopes da Silva & Van Rotterdam, 2005; Nunez & Silberstein, 2000).

2.2.2 EEG signal acquisition and processing

An EEG recording involves the measurement of fluctuating electrical field potentials in the brain across time (Kamp, Pfurtscheller, Edlinger, & Lopes da Silva, 2005). Electrodes are usually positioned on the scalp, in a location corresponding to a contemporary derivative of the Standardised International 10-20 system, which is based upon relative distance measurements using internationally recognised anatomical landmarks on the skull (Jasper, 1958; Klem, Luders, Jasper, & Elger, 1999). This standardised electrode placement allows for consistent interpretation of EEG recordings across laboratories. A suitable gel, paste or liquid is usually applied during electrode placement to assist with conduction (Rowan & Tolunsky, 2003) and

to reduce electrode to skin impedance, which can lead to distortions of the EEG signal (Teplan, 2002).

The amplitude of a typical adult scalp EEG signal ranges between approximately 10 and 100 µV in amplitude (Aurlien et al., 2004), and therefore needs to be greatly amplified before being transformed into a graphic representation that can be accurately measured and interpreted (Steven J. Luck, 2005; Rowan & Tolunsky, 2003). Signal at a given electrode represents the voltage difference between that electrode and the reference electrode signal (Steven J. Luck, 2005). There are several methods of providing a reference electrode signal, including the vertex electrode, mean recordings from electrodes positioned over bilateral mastoids, a common average reference representing the mean signal of all electrodes, or Laplacian data; a comparison between each electrode and the weighted average of the immediately surrounding electrodes (Nunez et al., 1997). During EEG recording, low-pass filters are used to attenuate undesirable high-frequency signals such as muscle potentials. High-pass filters are used to attenuate low frequency, slow potentials.

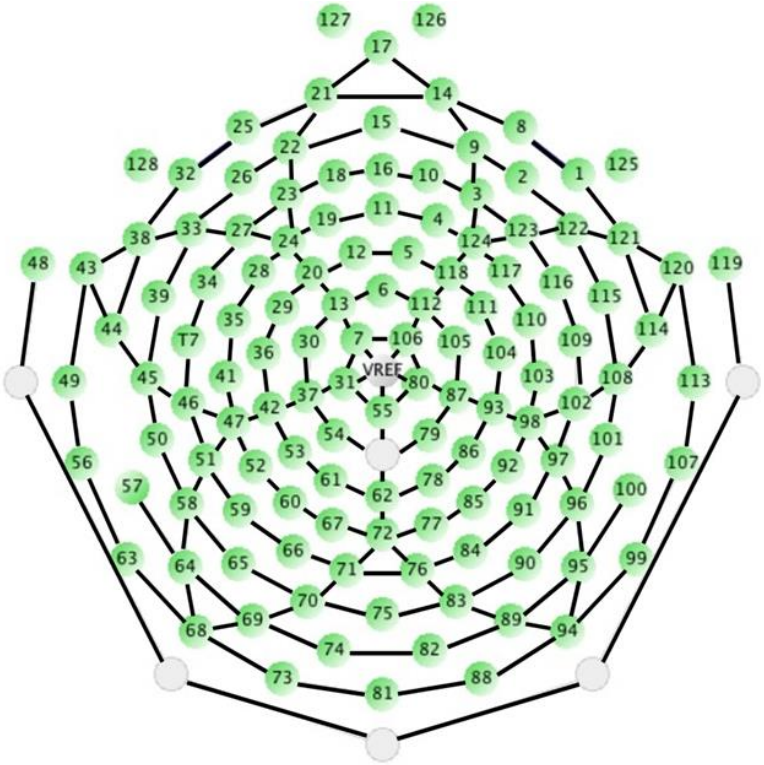

Figure 2.1:

Schematic of the 128 channel Geodesic sensor net. Electrode 17 is placed between the eyes, approximately 1 cm above the bridge of the nose. Electrodes 126 and 127 are cheek electrodes; electrodes 125 and 128 sit also on the cheeks, ventrally and caudally to 126 and 127.

Throughout the EEG recordings described in the following chapters, a 128-channel dense array net of sponge electrodes (Electrical Geodesics, Inc.) was used. The electrode net covered the entire vertex and back of the head, and much of the face (see Figure 2.1). A saline solution was used as a conductor, and the vertex electrode (VREF, commonly referred to as Cz) was used as the reference. Electrode-to-skin impedances were kept below 50 kΩ. A high-pass filter of 0.01 Hz, and a low-pass filter of 1000 Hz were employed.

2.2.3 Advantages and limitations of EEG recordings

A major advantage of EEG recording is its superb temporal resolution. Electrical changes that occur over the course of milliseconds can be detected (Schneider & Strüder, 2012), allowing for a direct read out of the processing of stimuli in real time. Specific aspects of sensory and cognitive processing, which can be more accurate and revealing than behavioural measures (e.g. reaction time) alone can be investigated (S. J. Luck, Woodman, & Vogel, 2000). A second advantage of EEG is that it provides a relatively direct measurement of neuronal activity in comparison to indirect haemodynamic responses recorded using fMRI or positron emission tomography (PET) (Hari, Parkkonen, & Nangini, 2010). EEG research is also practically advantageous as it is a non-invasive technique that can be carried out in a wide range of environments, and is considerably less expensive in comparison to fMRI, magnetoencephalography and PET (Schneider & Strüder, 2012).

The fundamental limitation of EEG investigation is poor spatial resolution in comparison to methods such as fMRI. Given that EEG is recorded from the scalp via electrodes, any electrical signal is attenuated by the tissues it must pass through, such as the meninges, cerebrospinal fluid and skull (Nunez et al., 1997). As a result, a definitive identification of the source of electrical activity is impossible. This is commonly referred to as the inverse problem. Complex mathematical algorithms are often used to reconstruct intracranial origins for a given EEG signal in source analysis methods; however, these are limited by the accuracy of conductivity models and brain templates (Schneider & Strüder, 2012).

2.2.4 Artifact rejection in EEG analysis

The amplification required to record electrocortical potentials also results in the amplification of extracerebral potentials many times their amplitude, which may render EEG uninterpretable. These extracerebral potentials include those caused by muscle movement, chewing, heart beat (electrocardiographic activity, ECG), eye blinks (electrooculagraphic activity, EOG) and eye movements, and are known as artifacts (Rowan & Tolunsky, 2003). Other artifacts can arise from electrode problems or electrical noise from alternating current electrical appliances causing a 50 Hz wavelength artifact in recordings. Some method of correction must be carried out to ensure that artifacts do not obscure the underlying EEG data. Trials containing artifacts can be manually disregarded following a visual inspection. Alternatively, principal component analysis (Berg & Scherg, 1994) or independent component analysis (Jung et al., 2000) techniques can be employed. These use mathematical algorithms to isolate the average EEG signal component responsible for a specific artifact (e.g. EOG or ECG artifacts), and subtract this component from the EEG signal to leave behind 'clean' data (Steven J. Luck, 2005).

2.2.5 Event-related potentials (ERPs)

The term 'event-related potential' (ERP) can be defined as time-locked EEG activity detected at electrodes following the onset of a sensory stimulus (Lopes da Silva, 2005). ERP responses to specific stimuli or events between groups or conditions are often compared in order to quantitatively analyse EEG data (Lopes da Silva, 2005). ERP responses from a large number of trials are required for successful analysis (Lopes da Silva, 2005). A sufficient number of ERP waveforms can be time-averaged to generate a robust mean waveform with positive and negative voltage deflections, referred to as components (Steven J. Luck, 2005). In cognitive neuroscience and psychophysiological research, ERP components are typically investigated using quantitative comparisons of latency or amplitude (Steven J. Luck, 2005). Strengths of ERP analysis overlap with those of EEG itself, the key advantage being excellent temporal resolution. One disadvantage of the ERP method is the

large number of trials required for successful quantitative analysis. Further, there is a risk that ongoing spontaneous activity could be misinterpreted as event-related data (Lopes da Silva, 2005).

2.2.6 ERP analysis using statistical parametric mapping (SPM)

In conventional ERP analysis, quantitative comparisons between experimental conditions are often performed on ERP components from individual electrodes during specific time windows that are predetermined by apriori hypotheses (Steven J. Luck, 2005). This conventional method may be adequate for research questions that have very specific hypotheses, or those investigating well established ERP components that have been isolated at certain electrodes in many previous studies, for example, the N170 component in face processing (Bentin, Allison, Puce, Perez, & McCarthy, 1996; Bentin & Deouell, 2000; Cauquil, Edmonds, & Taylor, 2000; Martin Eimer, 2000; Gauthier, Curran, Curby, & Collins, 2003; Itier & Taylor, 2004; Rossion, Dricot, et al., 2000; Rossion, Gauthier, et al., 2000). However, for more exploratory research questions in under-researched topics, there may not be enough evidence to justify analysing only one component at a single electrode. When there are no apriori hypotheses regarding when or where to look for an effect, statistical parametric mapping (SPM) may be a more appropriate tool for searching the whole brain across multiple time points for a given effect (Kiebel & Friston, 2004; Worsley, 2003). SPM is a mass univariate, voxel-based approach employing classical inference to interpret regionally specific responses to experimental factors in functional imaging (Friston, 2004; Friston et al., 1994). One analyses every voxel in the brain using any standard statistical test, and the resulting statistical parameters are assembled into an image, the statistical parametric map (Friston, 2004). Therefore, using SPM, EEG data from across all electrodes and time points during an epoch of interest can be investigated in a single model. In this way, a family of hypotheses can be tested without model refitting, and hypotheses that span multiple ERP components, or different parts of distinct ERP components can be tested. Hence, the SPM method facilitates a more exploratory approach to analysing spatiotemporal neuroimaging data (Kiebel & Friston, 2004).

In SPM maps, the value at each voxel is a statistic that expresses evidence against a null hypothesis of no experimentally induced activation (Friston et al., 1994). The SPM method therefore uses principles from Gaussian random field theory (Adler, 1981) to control for multiple comparisons. Further, degrees of freedom are adjusted for non-sphericity, which includes the variability in expression of ERPs over subjects and non-sphericity induced by experimental design (Kiebel & Friston, 2004). Hence, SPM provides a robust control over Type I error, yet remains sensitive to detect truly significant results (Poline et al., 1997). SPM software analyses ERP data in a two-stage, hierarchical process: The first level involves modelling and standard estimation of ERP effects within subject and trial type, and describes the observation for multiple ERPs. The second level models first level parameters among trial types and subjects that contain differences or treatment effects elicited by experimental design, allowing classical inference (using t- or F-statistics) about such effects using contrast vectors that specify a null hypothesis (Kiebel & Friston, 2004). Kiebel and Friston (2004) showed that the two-stage hierarchical model implemented by SPM results in a test more stringent than, and at least as sensitive as a conventional model for analysing EEG. The studies discussed in the following chapters therefore used SPM as a novel and exploratory approach to analysing ERPs.

2.3 Functional magnetic resonance imaging (fMRI) of the brain

2.3.1 Introduction and physics of MRI

Magnetic resonance imaging (MRI) is a safe, non-invasive technique used to generate images of body tissues for both clinical and research purposes (Mandeville & Rosen, 2002). Images are created through measurement of signal created by the activity of protons, typically found in H^+ hydrogen atoms (Mandeville & Rosen, 2002; Narashiman & Jacobs, 2002; Schild, 1992). Water contains two protons per molecule and is abundant in living tissues. In particular, water accounts for three quarters of brain weight (Mandeville & Rosen, 2002; Narashiman & Jacobs, 2002), and thus MRI is often used to create high quality images of the brain in both clinical and experimental settings.

Hydrogen protons act as tiny magnets, spinning about their axis with a positive charge (Pooley, 2005; Schild, 1992). In an MRI scan, participants lie supine in the scanner, which consists of an electric current flowing through wires immersed in liquid helium in the loop of a large superconducting magnet, producing a strong magnetic field (typically 1.5 or 3 Tesla) (Pooley, 2005). Protons in brain tissues align to create a net magnetisation parallel to this magnetic field (B_0), a process called longitudinal magnetisation (Hendee & Morgan, 1984; Pooley, 2005; Schild, 1992). Throughout the scan, radio frequency (RF) pulses are transmitted for short periods of time via a head coil fitted around the participants' head. RF pulses are delivered at a specific frequency, known as the larmor frequency, selected to target only appropriate nuclei (hydrogen protons). This phenomenon is known as resonance (Schild, 1992). RF pulses transfer energy to the protons, knocking them out of alignment, and rotate the net magnetisation into the transverse plane (transverse magnetisation) (Pooley, 2005; Schild, 1992). When the RF pulse is switched off, protons immediately begin to re-align with the static magnetic field (longitudinal relaxation). The time taken for protons to return to a longitudinal net magnetisation is known as T1 (Hendee & Morgan, 1984; Pooley, 2005; Schild, 1992). The RF pulse also causes the protons in body or brain tissues to precess in phase. When the pulse is switched off, this state relaxes and the protons move out of phase, known as transverse relaxation. The time taken for transverse relaxation is known as T2 (Pooley, 2005; Schild, 1992). T1 and T2 are independent processes that occur simultaneously; however, T2 can never exceed T1 (Hendee & Morgan, 1984; Pooley, 2005; Schild, 1992). Transverse magnetisation following the RF pulse induces an electrical current that is measured with a receiver coil inside the scanner, which is then digitised and recorded for later reconstruction of the MR signal (Hendee & Morgan, 1984; Pooley, 2005; Schild, 1992).

Longitudinal and transverse relaxation rates are different for protons associated with different tissues in the brain and body. In the brain, white matter has a short T1 and T2, CSF has a long T1 and T2, and grey matter has an intermediate T1 and T2. These differences are the fundamental source of contrast in T1- and T2-weighted MR images (Pooley, 2005; Schild, 1992). Imaging parameters can be manipulated to enhance the contrast between specific tissues, by altering the time to repeat (the time between RF pulses, TR) and the time to echo (the time between the

RF pulse and signal detection, TE). Short TR and TE result in T1-weighted images, where substances with a short T1, such as white matter, produce a stronger signal and appear brighter (Pooley, 2005; Schild, 1992). T1-weighted scans utilise superior spatial resolution and are typically used for high resolution structural imaging. Anatomical T1-weighted scans are routinely acquired in neuroimaging studies for clinical evaluation, and/or to accurately co-register findings from functional scans (Howarth, Hutton, & Deichmann, 2006). Conversely, long TR and TE produce T2-weighted images, where substances with a long T2 (e.g. CSF) appear brighter (Mandeville & Rosen, 2002; Pooley, 2005). T2-weighted images can be used to obtain functional MR data (Mandeville & Rosen, 2002).

2.3.2 Functional MRI and the blood-oxygen level dependent (BOLD) signal

In functional magnetic resonance imaging (fMRI), imaging parameters are manipulated to produce T2-weighted scans where the contrast is based on blood oxygenation, blood volume, or blood flow (Mandeville & Rosen, 2002). T2-weighted scans favour the imaging of water, and water molecules behave differently in the vicinity of paramagnetic fields. Contrast agents with paramagnetic properties can therefore be used to evaluate blood flow or volume changes in T2 scans (Narashiman & Jacobs, 2002). Deoxyhaemoglobin acts as an endogenous paramagnetic contrast agent, and attenuates the MR signal (S.-G. Kim & Bandettini, 2010; Mandeville & Rosen, 2002). Activation in brain structures due to task demands requires oxygen and glucose. This requirement results in increased cerebral blood flow that exceeds the cerebral metabolic oxygen utilisation rate, producing a surplus of oxygenated blood and a reduction in deoxyhaemoglobin (Fox & Raichle, 1986). Heightened oxygenation therefore produces an increase in BOLD signal intensity (Mandeville & Rosen, 2002). Hence, fMRI measures brain function indirectly through the blood-oxygen level dependent (BOLD) signal, which reflects the surplus of oxygenated blood in a brain region and thus regional activation (S. G. Kim & Ogawa, 2012). Several studies have shown that activity indicated by the BOLD signal closely relates to measured neuronal activity (Attwell & Iadecola, 2002; Ogawa et al., 2000; Rees, Friston, & Koch, 2000). However, one study using simultaneous fMRI and EEG recordings showed that BOLD signal correlated more

with local field potentials than with individual neuronal activity (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). How the BOLD signal relates to underlying neural activity is complex and still debated (Ekstrom, 2010).

In a typical fMRI scan, the brain is scanned at a low spatial resolution to allow for a rapid rate of image acquisition (Mandeville & Rosen, 2002). Images are acquired in slices which contain a uniform grid of data points (Lindquist, 2008). Slices are either separated by gaps of a few millimetres, or collected in an interleaved fashion (e.g. all odd-numbered slices followed by all even-numbered slices) to prevent crosstalk between protons bordering the edges of slices, which would reduce contrast (Hornak, 1996; Lipton, 2010; McRobbie, Moore, Graves, & Prince, 2007). The spatial information required to identify the location of the MR signal produced in both structural T1-weighted scans and functional T2-weighted scans is acquired by applying further magnetic fields, known as gradients (Narashiman & Jacobs, 2002). The brain is modelled as a 3D space containing around 100,000 voxels, known as 'k-space', defined by X, Y, and Z co-ordinates (Lindquist, 2008). Typically, in 3D imaging, slices are selectively excited in turn with a narrow slice selection gradient (Narashiman & Jacobs, 2002). Slice numbers are defined as the Y co-ordinate in k-space. Following this, a 'phase encoding' gradient is applied, where the duration and magnitude of the gradient can be manipulated to cause phasing/dephasing of spins that can then be measured to provide spatial information about the signal location. The phase encoding gradient is oriented perpendicularly to the slice selection gradient, producing the X co-ordinate in k-space. Finally, a uniform 'frequency encoding' gradient that is also dependent on location is applied. Spatial information is then mapped onto a frequency scale, and frequency encoding produces the Z co-ordinate (Narashiman & Jacobs, 2002). Hence, combinations of frequency encoding and phase encoding create 3D images in 'k space' (Narashiman & Jacobs, 2002). These are then reconstructed into brain images using complex algorithms such as the reverse Fourier transformation, which considers the intensity and location of signal in k-space using phase and frequency encoded data (Narashiman & Jacobs, 2002). Pulse sequences with specific combinations of RF frequency, gradient durations and magnitudes and frequency and phase encoding can be utilised to focus on specific aspects of an image (Narashiman & Jacobs, 2002).

In fMRI scans, the result of the above process is a 3D voxel map containing the BOLD signal intensity changes over time induced by a given task or stimulus (Lindquist, 2008). The BOLD response is convolved with the haemodynamic response function (HRF) to give regressors that are entered into the design matrix to produce statistical parametric maps with associated parameter estimates for each experimental condition over time (Friston, 2004). In this way, BOLD maps observed under varying experimental conditions can be compared and used to identify activations of cortical and subcortical brain areas induced by such conditions (Friston, 2004).

2.4 Summary

The present thesis used an olfactometer to induce pleasant and unpleasant experimental odours in EEG and fMRI experiments. Given the excellent temporal resolution of EEG and the superior spatial resolution of fMRI, collecting data from both allowed for a more complete investigation of the effects of odours on perception, and the associated cortical activations. Both EEG and fMRI data were analysed using SPM. As discussed above, SPM is a relatively novel approach towards ERP analysis, and suited the exploratory nature of the studies in the present thesis.

Chapter 3

Pleasant and unpleasant odours influence hedonic evaluations of human faces: an event-related potential study.

This experiment investigated the effects of pleasant and unpleasant odours on evaluations of faces, using EEG.

It is published in Frontiers in Human Neuroscience (2015), doi: 10.3389/fnhum.2015.00661. The format and parts of the content have been altered to match the style of the thesis.

The roles of the co-authors are summarised below:

I designed the study in collaboration with Andrej Stancak and collected the data. Nicholas Fallon and Hazel Wright assisted with the data collection. Andrej Stancak and Nicholas Fallon provided training on the experimental set-up and data analysis. I analysed the data, interpreted the results, and wrote the manuscript. Nicholas Fallon, Hazel Wright, Anna Thomas, Timo Giesbrecht, Matt Field, and Andrej Stancak contributed useful comments while preparing the manuscript for publication.

3.1 Abstract

Odours can alter hedonic evaluations of human faces, but the neural mechanisms of such effects are poorly understood. The present study aimed to analyse the neural underpinning of odour-induced changes in evaluations of human faces in an odour-priming paradigm, using event-related potentials (ERPs).

Healthy, young participants (N = 20) rated neutral faces presented after a three second pulse of a pleasant odour (jasmine), unpleasant odour (methylmercaptan), or no-odour control (clean air).

Neutral faces presented in the pleasant odour condition were rated more pleasant than the same faces presented in the no-odour control condition, which in turn were rated more pleasant than faces in the unpleasant odour condition. Analysis of face-related potentials revealed four clusters of electrodes significantly affected by odour condition at specific time points during long-latency epochs (600−950 ms). In the 620−640 ms interval, two scalp-time clusters showed greater negative potential in the right parietal electrodes in response to faces in the pleasant odour condition, compared to those in the no-odour and unpleasant odour conditions. At 926 ms, face-related potentials showed greater positivity in response to faces in the pleasant and unpleasant odour conditions at the left and right lateral frontal-temporal electrodes, respectively.

Our data shows that odour-induced shifts in evaluations of faces were associated with amplitude changes in the late (> 600 ms) and ultra-late (> 900 ms) latency epochs. The observed amplitude changes during the ultra-late epoch are consistent with a left/right hemisphere bias towards pleasant/unpleasant odour effects. Odours alter evaluations of human faces, even when there is a temporal lag between presentation of odours and faces. Our results provide an initial understanding of the neural mechanisms underlying effects of odours on hedonic evaluations.

3.2 Introduction

A number of behavioural studies have investigated cross-modal effects of odours on evaluations of human faces (Dematte et al., 2007; Leppanen & Hietanen, 2003; Li et al., 2007; McGlone et al., 2013; Seubert et al., 2014; Todrank et al., 1995). In general, pleasant odours increased preferences for faces, with unpleasant odours having the opposite effect. The neural mechanisms that underlie such effects are not yet established. One study found that repeated pairing of emotionally neutral faces with pleasant and unpleasant odours resulted in conditioned shifts in face ratings (when presented subsequently, without odours), but failed to show any significant cortical changes related to conditioning (Herrmann et al., 2000). Another study paired pleasant and unpleasant odours with positively and negatively valenced facial expressions, demonstrating evaluative changes that occurred as a function of hedonic congruency between the odour prime and target face and increased late-positive potential (LPP) amplitude for incongruent odour-face pairings (Bensafi, Pierson, et al., 2002). However, neural processes underlying immediate odour-induced changes in evaluations of emotionally neutral faces, where evaluative congruency or conditioned pairing does not play a role, remain unknown.

Most previous studies investigating effects of odours on immediate evaluations of faces used paradigms where the odour primes and target faces overlapped (Dematte et al., 2007; Leppanen & Hietanen, 2003; Seubert et al., 2014), or where target faces appeared at the offset of the odour prime (Bensafi, Pierson, et al., 2002). This complicates interpretation of the findings, because any shift in target evaluation could be attributable to affective responses to the odours themselves (Herring et al., 2013). It is important to establish whether or not odour-related evaluative shifts can survive after inserting a temporal lag between odour primes and target faces. This should ensure unbiased shifts in evaluative ratings that occur as a result of priming effects activated by the odour valences, which then carry over to the evaluation of the target face.

The aim of the present study was to investigate the neural underpinning of odour-induced changes in immediate hedonic evaluations of neutral faces, by observing the influence of both pleasant and unpleasant odours on evaluations of

emotionally neutral male and female faces that were presented one second after odour offset. We used a novel and exploratory approach to analyse odour-induced modulations in the ERP response to faces. Based on the previous literature, we hypothesised that faces in the pleasant odour condition would be rated as most pleasant, faces in the unpleasant odour condition would be rated as least pleasant, and faces in the clean air condition would be rated in between the two. We also hypothesised that odour-induced change in the ERP response to faces would be reflected in the LPP.

3.3 Methods and materials

3.3.1 Participants

A total of 23 (11 male) participants aged 18−36 years (mean ± standard deviation: 24.65 ± 4.35) were screened in a session prior to the experiment after responding to the study advertisement. All but four participants were right-handed. People suffering from asthma or neurological disorders, particularly anosmia or epilepsy, were not permitted to take part in the study. Normal olfactory function was ascertained using the Sniffin'Sticks (Hummel, Sekinger, Wolf, Pauli, & Kobal, 1997) test battery. Participants had to successfully identify a minimum of 9 out of the 12 odours in order to take part in the experiment. The mean score on the Sniffin'Sticks odour identification task was 10.5 (± 1.5). Three people were excluded from participation at the screening stage after scoring below 9 on the Sniffin'Sticks task. Hence, a total of 20 participants (mean age: 25.15 ± 4.43) participated in the experiment. Participants were asked not to smoke, drink coffee or chew gum for two hours prior to the experiment, and were asked to minimise their use of fragranced products on the day. Participants were reimbursed for their time and travel expenses. The study was approved by the Research Ethics Committee at the University of Liverpool. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

3.3.2 Visual and olfactory stimuli

A total of 36 (18 male) neutral faces obtained from the NimStim Set of Facial Expressions (Tottenham et al., 2009) were used in the experiment. Out of the 18 female faces, 9 were white/Caucasian, 5 were East-Asian, and 4 were Afro-Caribbean. Out of the 18 male faces, 12 were white/Caucasian, 5 were afro-Caribbean and 1 was East-Asian. Participants were gathered from a student population at the University of Liverpool, and were therefore a mixture of races and ethnicities with a white/Caucasian majority. Data on the race/ethnicity of participants was not recorded for ethical reasons. All face images were frontal views, in colour, with a consistent light background. All images measured 253 × 312 pixels. During the screening session, participants rated the perceived pleasantness of the facial expressions of all 36 faces (on a scale ranging from 0 – very unpleasant to 100 – very pleasant) in order to ensure that they were perceived as neutral. The mean face pleasantness rating was 47.80 (± 7.2).

Odours were administered through two tubes approximately two centimetres away from the nostrils; using a custom-built, continuous airflow, computer-controlled olfactometer with 8 channels (Dancer Design Ltd., UK). Odour pulses were embedded within a constant flow of clean air, in order to avoid effects of a sudden increase in airflow associated with presentation of an odour (Huart et al., 2012). Airflow was kept constant at approximately 2.2 l/min.

There were three odour conditions in the experiment; pleasant, unpleasant and a neutral control. Methylmercaptan (1% dilution in Propylene Glycol), a rotten smelling odour, was selected for the unpleasant condition. Jasmine odour (no dilution) was selected for the pleasant condition. These odours were from a small sample of odours recommended by Unilever; selected on the basis that they were not food-specific, and were quickly and accurately recognised as very pleasant and very unpleasant. A previous study showed that odours affect ratings of faces, regardless of whether or not the odours were body relevant (Dematte et al., 2007). Although Jasmine and Methylmercaptan compounds have no direct body relevance, Jasmine may have been more likely to influence evaluations of faces than other odours because it is commonly used in perfumes. However, most pleasant, non-food-specific

odours are likely found in perfumes. Moreover, although jasmine odour is typically perceived as pleasant by the majority of people, in its natural form, it may contain around 6% indole, a pure chemical which is usually perceived as unpleasant (Grabenhorst, Rolls, & Margot, 2011; Grabenhorst et al., 2007). However, the jasmine odour used in the present experiments contained just 0.024% indole, and moreover, previous studies showed that jasmine with and without indole were both rated as pleasant, and did not differ significantly in terms of pleasantness ratings (Grabenhorst et al., 2011; Grabenhorst et al., 2007). Hence, the chances of such a concentration of indole affecting the influence of the jasmine odour in the present experiments are slim to none. Odour dilutions were matched on perceived intensity based on data from a pilot study carried out on a separate sample prior to the experiment (N = 15). Odours were supplied by Symrise Ltd. (Netherlands). Propylene Glycol (1,2-Propanediol 99%, Sigma-Aldrich Ltd., UK) was used for dilution, the clean air control and constant flow.

Both presentation of the visual task stimuli and triggering of the odour valves was accomplished using Cogent software for Matlab (MATLAB v. R2011a program, The MathWorks, Inc., USA). In between experimental blocks and sessions, a Blueair 203 air purifier (Blueair Ltd., Sweden) was used to minimise any residual odour that may have carried into the next experimental block or session.

3.3.3 Recordings

EEG was recorded continuously using a 128-channel Geodesics EGI System (Electrical Geodesics, Inc., Eugene, Oregon, USA) with the sponge-based Geodesic Sensor Net. The sensor net was aligned with respect to three anatomical landmarks; two pre-auricular points and the nasion. Electrode-to-skin impedances were kept below 50 kΩ and at equal levels across all electrodes. The recording band-pass filter was 0.01−1000 Hz, and the sampling rate was 1000 Hz. Electrode Cz was used as the reference.

Participants' respiration and pulse rate were recorded continuously throughout the experiment with a piezoelectric respiratory belt transducer worn

around the chest at the level of the epigastrium, and a finger pulse oximeter transducer worn on the index finger of the left hand (ADInstruments Ltd., Oxford, UK). Signals were transduced and extracted using LabChart 7 (ADInstruments Ltd., Oxford, UK).

3.3.4 Procedure

After application of the EEG cap, participants were seated in a dimly lit, sound attenuated room with a 19 inch CRT monitor (60 Hz refresh rate) placed 0.7 m in front of them. First, the respiratory and pulse monitoring equipment was fitted onto participants and the signals were checked. Following this, the olfactometer head piece was fitted, and participants were given some instructions. The experimental session lasted around one hour in total, including baseline odour ratings and the experimental task. Ratings of odour pleasantness, intensity and familiarity were recorded before and after the task. Odours were administered individually, in a four-second pulse manually triggered to coincide with the onset of inspiration. After each odour pulse, on-screen visual analogue scales prompted participants to rate the pleasantness (from 0 – very unpleasant to 100 – very pleasant), intensity (0 – no odour to 100 – very intense odour) and familiarity (0 – not familiar at all to 100 – extremely familiar) of the odour.

The experimental task was split into three blocks of 36 trials. Trials were pseudo-randomly ordered, such that each of the 36 faces used in the task appeared only once in each block, and once with each odour. Odour presentation was also pseudo-random, such that all three odours were presented across all three blocks, but no two consecutive trials used the same odour. Figure 3.1 shows a flowchart of the trial procedure. Each trial began with a resting interval during which subjects viewed a white cross on a black background. Duration of this interval was dependent upon the triggering of the odour pulse; the experimenter observed participants' respiratory waveforms, and manually triggered the odour pulses at the very onset of inspiration. A three second odour pulse was then released, during which participants viewed a black screen. The screen remained black for a further one second resting interval, before a neutral face was displayed on-screen for 300 ms. Following this, a 1700 ms

resting interval with a black screen preceded a rating scale prompting participants to rate the pleasantness of the neutral face (from 0 – very unpleasant to 100 – very pleasant). Once participants had responded, a second scale prompted them to rate the intensity of the odour administered in that trial (0 – no odour to 100 – very intense odour). After their response, the next trial began.

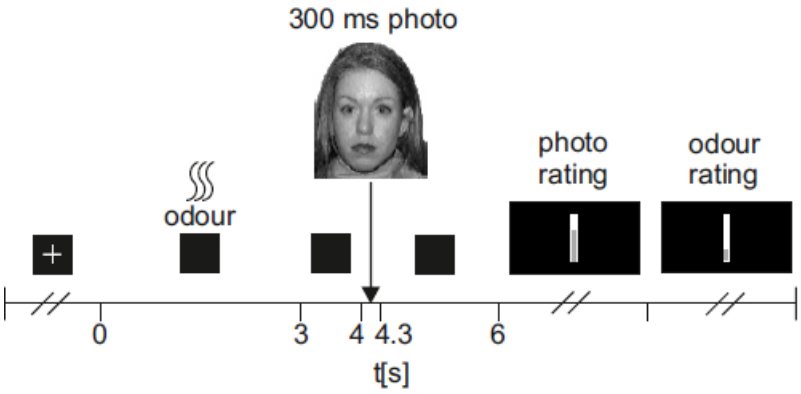

Figure 3.1: Flowchart of experimental trial procedure.

3.3.5 Behavioural analysis

Ratings of odour pleasantness, intensity and familiarity were analysed using 3 × 2 repeated measures ANOVAs. The independent variables were odour condition (clean air, methylmercaptan and jasmine), and time (before/after priming task). Data from the experimental task were analysed using one-way ANOVAs, observing differences in face pleasantness ratings and odour intensity ratings across the three odour conditions. Two-way ANOVAs were used to investigate effects of gender and experimental block on face pleasantness and odour intensity ratings. Significant main effects were investigated using pairwise comparisons; significant interactions were followed up with post-hoc t-tests. All behavioural data were analysed using SPSS v. 22 software package (IBM Inc., USA).

3.3.6 ERP analysis

EEG recordings were pre-processed using BESA v. 6.0 (MEGIS GmbH, Germany). Data were first referenced to a common average using common averaging method (Lehmann, 1987). The oculographic and, when necessary, electrocardiographic artifacts were removed by principal component analysis (Berg & Scherg, 1994). Data were visually inspected for the presence of any movement or muscle artifacts, and epochs contaminated with artifacts were excluded. The average numbers of accepted trials in each condition were as follows: clean air, 33.75 (± 2.07); jasmine, 33.65 (± 1.75); methylmercaptan, 32.9 (± 1.68). The average number of trials accepted did not differ across conditions ($P > 0.05$).

Data were band-pass filtered from 0.5−30 Hz and down-sampled to a rate of 256 Hz, and exported from BESA into the SPM12 software package (Statistical Parametric Mapping, UCL, England; http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Event-related potentials (ERPs) in response to neutral faces were computed separately for each odour condition by averaging respective epochs in the intervals ranging from 300 ms before photo onset to 1200 ms after photo onset. The baseline period ranged from -300 ms to 0 ms relative to the onset of the visual stimulus. Grand average waveforms were computed.

Face processing spans over multiple ERP components (Bentin et al., 1996; Cacioppo, Crites, Berntson, & Coles, 1993; Duval, Moser, Huppert, & Simons, 2013; Hajcak, Dunning, & Foti, 2007; Hajcak, Moser, & Simons, 2006; Rossion & Jacques, 2011). Relatively subtle effects of odours on hedonic aspects of face perception would likely involve late potential components, such as the late-positive potential (LPP) known to operate in a long latency window from 600 ms to 2000 ms (Cacioppo et al., 1993; Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Duval et al., 2013; Hajcak et al., 2007; Hajcak, MacNamara, & Olvet, 2010; Hajcak et al., 2006; Hajcak & Olvet, 2008; MacNamara & Hajcak, 2010; Weinberg & Hajcak, 2010). The late potential components do not show a distinct potential peak allowing for a traditional ERP analysis in which ERP data would be reduced to only a small number of components based on their peak latencies. Therefore, we applied an

omnibus analysis of effects of odours on ERPs involving all time points from 0 ms to 1000 ms and all scalp sites, allowing us to explore effects of odours on ERPs without applying a priori knowledge of peak latencies. The Statistical Parametric Mapping (SPM) software combines advanced statistical models with robust control for Type I error (Poline, Holmes, Worsley, & Friston, 1997; Kiebel & Friston, 2004). In contrast to alternative approaches, such as permutation analysis of clusters of ERPs over the epoch time (Maris & Oostenveld, 2007), SPM applies the theory of random fields to the volumes of space-time data which allows to calculate the degrees of freedom in evaluation of statistical test results based on the spatial and temporal complexity of data (Worsley, 2003).

The statistical analysis was performed in two steps. In the initial exploratory step, EEG data were converted into three-dimensional scalp-time images using SPM. The electrodes were mapped onto a standardised scalp grid sized 32 × 32 pixels (pixel size 4.25 × 5.3 mm^2), representing the field potential planes stacked over the time axis. Images were smoothed with a Gaussian kernel of 9 × 9 × 20 mm^2 .ms (full width at half maximum). Data from over the whole epoch (385 time samples) and all standardised scalp points were screened for a statistically significant effect of odours using a one-way ANOVA for repeated measures. We applied a liberal, uncorrected threshold of P = 0.005 and a cluster size threshold of 20 contiguous space-time voxels to detect clusters significantly affected by odours. The amplitude data from these clusters were subsequently analysed using further one-way ANOVA for repeated measures in SPSS v. 22 (IBM Inc., USA). The statistical threshold of this confirmatory analysis was P = 0.05.

3.3.7 Analysis of respiratory movements

Respiratory signals were low-pass filtered and averaged separately for each of the three odour conditions, then analysed statistically using a one-way ANOVA in Matlab. The 10 s analysis window ranged from 3 s before to 7 s after onset of odour, with the interval 7100-8100 ms overlapping with the ERP analysis epoch. A permutation analysis with 2000 permutations was used to correct the P values. We used a one-way ANCOVA for repeated measures in BMDP 2V program (Biomedical

Data Package, Cork, Ireland) to analyse whether changes in respiratory movement patterns contributed to the effects of odours seen in ERP clusters.

3.4 Results

3.4.1 Odour ratings

Table 3.1 shows the mean ratings of odour pleasantness, intensity and familiarity before (Time 1) and after (Time 2) the priming task. A repeated-measures ANOVA confirmed a significant main effect of odour type on pleasantness ratings across both time points ($F(2,38) = 95.93$, η_p^2 (partial eta square) = 0.84, $P < 0.001$). Overall, jasmine was rated as most pleasant (76.20 ± 16.6), methylmercaptan as least pleasant (12.31 ± 15.37), and clean air was rated close to neutral (55.22 ± 10.78). Pairwise comparisons indicated that all three odours significantly differed from each other in terms of pleasantness ($P < 0.001$). There was no main effect of time (before/after task), or interaction between time and odour affecting pleasantness ratings ($P > 0.05$), suggesting that perceptions of odour pleasantness remained stable throughout the experiment.

Table 3.1: Mean (± standard deviation) ratings of odour pleasantness, intensity and familiarity taken before and after the task.

	Pleasantness		Intensity		Familiarity	
	Time 1	Time 2	Time 1	Time 2	Time 1	Time 2
Clean Air	54.07 (± 8.16)	56.36 (± 13.23)	13.02 (± 19.5)	2.27 (± 3.8)	80.72 (± 23.53)	84.95 (± 17.94)
Jasmine	74.7 (± 13.15)	77.72 (± 20.05)	62.8 (± 16.27)	74.31 (± 15.91)	63.82 (± 24.61)	72.25 (± 24.3)
Methylmercaptan	13.5 (± 14.12)	11.13 (± 17.19)	84.95 (± 8.4)	83.21 (± 15.44)	52.3 (± 29.5)	61.8 (± 32.93)

A repeated-measures ANOVA revealed a significant main effect of odour on intensity ratings across both time points ($F(2, 38) = 318.41$, $\eta_p^2 = 0.94$, $P < 0.001$). Pairwise comparisons indicated that jasmine was perceived as significantly more intense (68.6 ± 16.71) than clean air (7.64 ± 14.7; $P < 0.001$). In spite of pilot data suggesting that the jasmine and methylmercaptan odours were matched for perceived intensity, pairwise comparisons showed that methylmercaptan was perceived as significantly more intense (84.08 ± 12.2) than both jasmine ($P < 0.001$) and clean air ($P < 0.001$) across both time points. There was no main effect of time on intensity ratings; however there was an interaction between time and odour affecting intensity ratings ($F(2, 38) = 10.18$, $\eta_p^2 = 0.35$, $P < 0.001$). Post-hoc t-tests were employed to further investigate this interaction. These confirmed that clean air was perceived as less intense at Time 2 (after the priming task) in comparison to Time 1 (before the priming task) ($t(19) = 2.61$, $P = 0.02$). Further, jasmine was perceived as more intense at Time 2 in comparison to Time 1 ($t(19) = -2.83$, $P = 0.01$). There was no significant difference in intensity ratings of methylmercaptan across time points ($P > 0.05$).

A repeated-measures ANOVA confirmed a significant main effect of odour on familiarity ratings across both time points ($F(2, 38) = 7.91$, $\eta_p^2 = 0.29$, $P = 0.001$). Pairwise comparisons indicated that clean air was rated as more familiar (82.83 ± 20.51) than both jasmine (68.03 ± 24.20; $P = 0.02$), and methylmercaptan (57.04 ± 30.82; $P = 0.004$). There was no difference in familiarity ratings of jasmine and methylmercaptan ($P > 0.05$), and there was no main effect of time, or interaction between time and odour affecting familiarity ratings ($P > 0.05$).

3.4.2 Face and odour ratings during the experiment

Table 3.2 shows the mean pleasantness ratings of faces under each odour condition. A one-way ANOVA revealed a significant effect of odour on pleasantness ratings of faces ($F(2,38) = 13.41$, $\eta_p^2 = 0.41$, $P < 0.001$). Pairwise comparisons indicated that neutral faces were rated as more pleasant after presentation of the jasmine odour in comparison to faces in both the clean air ($t(19) = 3$, $P = 0.007$) and

methylmercaptan (t(19) = 4.16, P = 0.001) conditions; and faces in the methylmercaptan condition were rated as significantly less pleasant than those in the clean air condition (t(19) = -3.09, P = 0.006).

Table 3.2: Mean (± standard deviation) pleasantness ratings of neutral face photographs and odour intensity ratings under three odour conditions during the experimental task.

	Face rating	Odour intensity rating
Clean Air	53.19 (± 4.1)	5.6 (± 7.05)
Jasmine	55.26 (± 4.3)	56.33 (± 15.83)
Methylmercaptan	50.19 (± 3.92)	61.34 (± 17.68)

We analysed whether odours affected pleasantness ratings of faces differently in male and female participants. A two-way mixed ANOVA (male vs. female participants, three odours) showed no significant effect of participant gender on face ratings (P > 0.05). Importantly, there was no significant interaction between odour and gender affecting face ratings (P > 0.05), and therefore data were analysed further without splitting them based on the gender factor.

We also evaluated effects of experimental block on effects of odours on face pleasantness ratings. The statistical analysis consisted of two-way ANOVAs with three odours and three experimental blocks as independent variables. There was an interaction between odour and block affecting face pleasantness ratings (F(4,76) = 4.95, η_p^2 = 0.2, P = 0.003). Post-hoc one-way ANOVAs showed that in the pleasant odour condition, there was a significant effect of block (F(2,38) = 5.27, η_p^2 = 0.22, P = 0.14), with pairwise comparisons indicating that faces presented in the pleasant odour condition were rated as more pleasant in block 2 of the experiment in comparison to both block 1 (P = 0.05) and block 3 (P = 0.001). In the unpleasant odour condition, the effect of block was statistically significant (F(2,38) = 6.15, η_p^2 = 0.25, P = 0.006). Pairwise comparisons indicated that faces presented in the unpleasant odour condition were rated as less pleasant in blocks 2 and 3 in

comparison to when they were presented in block 1 (P = 0.008 and P = 0.017, respectively).

Table 3.2 also shows the mean odour intensity ratings for each odour condition. A one-way ANOVA revealed a significant effect of odour on intensity ratings ($F(2, 38) = 180.74$, $\eta_p^2 = 0.91$, $P < 0.001$). Pairwise comparisons indicated that both jasmine ($t(19) = -15.51$, $P < 0.001$) and methylmercaptan ($t(19) = -14.34$, $P < 0.001$) were rated as significantly more intense than clean air. There was no significant difference between intensity ratings of jasmine and methylmercaptan ($t(19) = -2.08$, $P > 0.05$).

Odour intensity ratings also changed over the course of the experiment ($F(2,38) = 11.62$, $\eta_p^2 = 0.38$, $P = 0.001$). Pairwise comparisons indicated that all odours were rated as most intense during block 1 (mean ± SE 45.42 ± 2.16), and least intense during block 3 (37.44 ± 2.87). Odours in block 2 were rated in between the two (40.05 ± 2.91) ($P < 0.05$). However, there was no significant interaction between odour and block affecting odour intensity ratings ($P > 0.05$).

3.4.3 ERP components

Figure 3.2 illustrates the event-related potentials in response to faces across all trials and all odour conditions in the form of a butterfly plot and topographic maps of selected potential components. Topography of the first component showed bilateral positivity over the occipital electrodes and negativity over frontal electrodes, peaking around 135 ms, consistent with characteristics of the P1 component − related to early processing of visual stimuli (Hopf, Vogel, Woodman, Heinze, & Luck, 2002). Further, the second component, peaking around 175 ms, showed strong negativity over posterior parietal and temporal electrodes, consistent with characteristics of the N170 face-processing component (Bentin et al., 1996).

The next component peaked around 250 ms, showing strong positivity over occipital/parietal electrode sites, consistent with the P300 component, which is involved in information-processing in attentional and memory mechanisms (Polich, 2012). The fourth component was similar, peaking at approximately 430 ms and

showing negativity over centro-parietal electrode sites; consistent with the N400 component, which is implicated in the processing of meaningful stimuli, including faces (Kutas & Federmeier, 2011).

A further component was a long component beginning around 500 ms and peaking at approximately 570 ms. Showing negativity over occipital electrode sites and positivity over parietal areas, it had a similar topography to the N170 and was consistent with characteristics of the late positive potential (LPP) which is sensitive to the emotional content of pictures, words and faces (Cacioppo et al., 1993; Cuthbert et al., 2000; Hajcak et al., 2007; Hajcak et al., 2006). The final component was a second long-latency component, beginning around 650 ms and extending until 1000 ms, it peaked around 810 ms and showed negativity over the right temporal-parietal electrodes, and positivity over frontal electrodes. These two late components are comparable with the mid- and late-LPP components observed in a recent study investigating ERPs in response to faces (Duval et al., 2013).

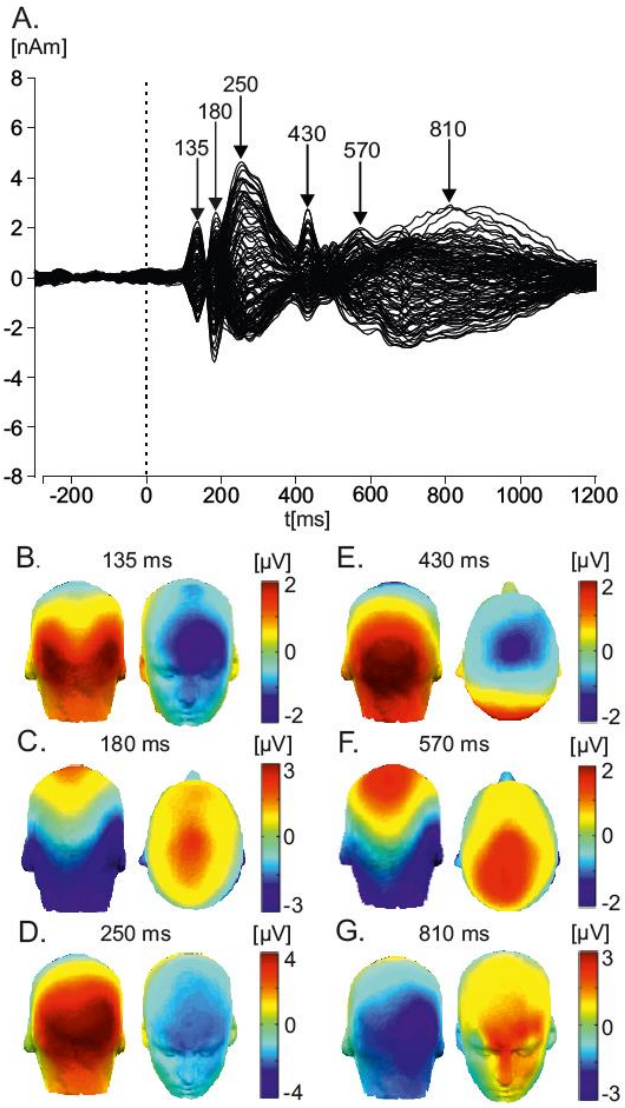

Figure 3.2: Butterfly plot of grand average ERP responses to faces and corresponding scalp topographies. (A) Butterfly plot of grand average ERP responses to faces. Peak latencies of distinct ERP components (135 ms, 180 ms, 250 ms, 430 ms, 570 ms, and 810 ms) are highlighted with arrows. (B) Latency component 135 ms (P1). The topographic maps of grand average ERPs overlaid on the volume rendering of the human head are shown. (C) Latency component 180 ms (N170). (D) Latency component 250 ms (P300). (E) Latency component 430 ms (N400). (F) Latency component 570 ms (late component/LPP). (G) Latency component 810 ms (ultra-late component/LPP).

3.4.4 Effects of odours on ERPs

SPM12 software was used to compute a one-way ANOVA on smoothed scalp-time images of data from 0−1000 ms relative to onset of the face. The one-way ANOVA revealed four scalp-time clusters that showed significant effects of odour. Amplitude data from each of these scalp-time clusters was then extracted, and further one-way ANOVAs were computed on the data using SPSS. Figure 3.3 illustrates these significant scalp-time clusters. The corresponding topographic maps from each odour condition for each significant cluster are shown with bar graphs showing the mean EEG scalp-amplitude (μV).

At 621 ms and 633 ms following onset of the face photograph, there was a significant effect of odour in the right parietal electrodes. Given that the two clusters were within 20 ms of one another, it is likely that they reflect a similar process. In a preliminary analysis, we analysed the amplitude data from these two clusters in a two-way ANOVA, with odour and cluster as independent variables. There was no significant effect of cluster, or interaction between odour and cluster affecting amplitude ($P > 0.05$). Therefore, we chose to average the amplitude data from the two clusters. There was a significant effect of odour on the averaged amplitude data from clusters at 621 ms and 633 ms following onset of the face ($F(2,38) = 7.89$, $\eta_p^2 = 0.29$, $P = 0.001$). Pairwise comparisons indicated a significantly stronger negative amplitude for faces presented after administration of the jasmine odour in comparison to those in both the clean air ($P = 0.01$) and methylmercaptan ($P = 0.001$) conditions. There was no significant difference in amplitude between the clean air and methylmercaptan conditions ($P > 0.05$).

At 926 ms following the onset of the face, there were two significant clusters; one in the left hemisphere ($F(2,38) = 4.84$, $\eta_p^2 = 0.2$, $P = 0.014$), and one in the right hemisphere ($F(2,38) = 4.72$, $\eta_p^2 = 0.2$, $P = 0.026$), both at lateral fronto-temporal electrode sites. Pairwise comparisons indicated that in the left hemisphere, the positive amplitude was significantly greater in the jasmine condition compared to the methylmercaptan condition ($P = 0.003$). Amplitude differences between the jasmine and clean air, and clean air and methylmercaptan conditions were non-significant (P

> 0.05). In the right hemisphere, there was significantly greater positive amplitude in the methylmercaptan condition in comparison to the jasmine condition (P = 0.009). The amplitude difference between the clean air and jasmine conditions was also significant (P = 0.02), but there was no significant difference in amplitude between the clean air and methylmercaptan conditions (P > 0.05).

Pearson correlation analyses were computed with amplitude data from each significant scalp-time cluster (621 ms, 633 ms, and 926 ms – left and right hemisphere), baseline odour pleasantness and intensity ratings (taken before and after the task), face ratings throughout the task, and odour intensity ratings throughout the task, for both pleasant and unpleasant odour conditions. Table 3.3 shows Pearson's *r* correlation coefficients and statistical values for bivariate correlations between amplitude data and subjective ratings. Of these correlations, one remained significant after applying Bonferroni-Šidák correction for multiple comparisons. Odour pleasantness ratings, and left hemisphere potential amplitude at 926 ms in the unpleasant odour condition were negatively correlated (r(20) = -0.62, P = 0.003). Baseline intensity ratings and left-hemisphere amplitude at 926 ms in the pleasant odour condition were positively correlated, but only borderline significant after Bonferroni-Šidák correction, (r(20) = 0.56, P = 0.01). No correlations between amplitude data and photo/odour ratings throughout the task reached significance (P > 0.05).

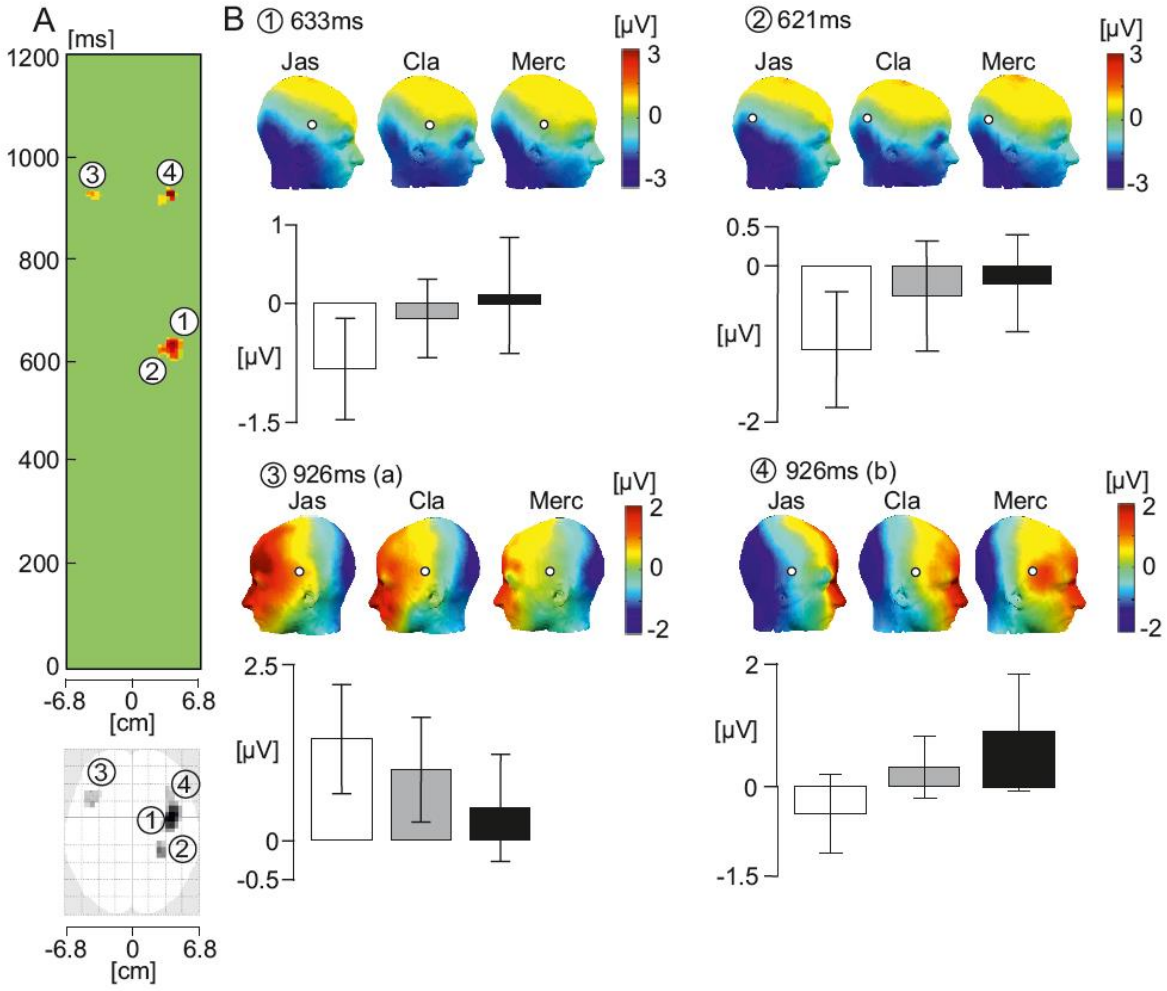

Figure 3.3: One-way ANOVA showing the effects of the three odour conditions on ERP responses to faces. (A) The green panel shows statistically significant latency periods (uncorrected $P < 0.005$) in the scalp-time plot where F values represent the strength of variance between odour conditions over the horizontal axis of the scalp in every time sample from 0 ms and 1200 ms relative to the onset of the face photograph. The scalp values over the horizontal axis of the scalp are averages of F values occurring at each vertical point for a given horizontal point in the standardised scalp map (from -6.8 cm to +6.8 cm). Two latency intervals showed the presence of statistically significant spatio-temporal clusters. In the interval 600−640 ms, two clusters numbered 1 and 2 showed a significant effect of odour condition. In the latency period 910−930 ms, clusters numbered 3 and 4 showed a significant effect of odour condition. Below the green panel is the standard scalp map of statistically significant clusters using ERPs. (B) Corresponding topographic maps of the numbered significant cluster latencies under each odour condition (Jas – jasmine, pleasant odour; Cla – clean air, control; Merc – methylmercaptan, unpleasant odour). White circles with a black outline pinpoint the location of the significant electrode clusters. Bar graphs below illustrate the mean EEG amplitude for each cluster/latency under each odour condition (μV). White bars represent the pleasant odour condition, grey bars represent the neutral control condition, and black bars represent the unpleasant odour condition.

Table 3.3: Pearson correlations (*r* and P values) for amplitude data at each significant scalp-time cluster and baseline ratings of odour pleasantness and intensity, photograph ratings and odour intensity ratings throughout the task for both pleasant and unpleasant odour conditions. Correlation is significant at the P < 0.05 level (two-tailed) following Bonferroni- Šidák correction for multiple tests. 926 ms[a] and 926 ms[b] represent clusters at 926 ms in the left and right hemispheres, respectively.

Pleasant odour

Cluster	Baseline pleasantness		Baseline intensity		Photo rating		Odour rating	
	r	P	*r*	P	*r*	P	*r*	P
621 ms	-0.22	0.36	0.05	0.84	0.35	0.13	-0.34	0.14
633 ms	-0.42	0.86	0.11	0.64	0.02	0.93	0.06	0.81
926 ms[a]	0.39	0.08	0.56	0.01*	-0.28	0.23	-0.05	0.84
926 ms[b]	-0.41	0.07	-0.13	0.59	0.23	0.34	0.06	0.79

Unpleasant odour

Cluster	Baseline pleasantness		Baseline intensity		Photo rating		Odour rating	
	r	P	*r*	P	*r*	P	*r*	P
621 ms	-0.11	0.65	-0.01	0.99	-0.15	0.52	-0.83	0.73
633 ms	-0.12	0.61	0.07	0.76	-0.19	0.42	-0.01	0.99
926 ms[a]	-0.62	0.003**	0.11	0.65	0.35	0.13	-0.04	0.86
926 ms[b]	0.19	0.41	-0.09	0.68	-0.05	0.85	-0.22	0.35

3.4.5 Analysis of respiratory movements

Figure 3.4 shows averaged respiratory waveforms for each odour condition in a 10 second interval, beginning three seconds prior to odour onset. Odours significantly affected respiratory movements in two intervals, one 5000−5800 ms, and another 7100−8100 ms. The latter interval overlapped with the period in which ERPs were recorded and analysed. In both intervals showing a statistically significant effect of odours, the respiratory movements in the clean air condition differed from both pleasant and unpleasant odour conditions. However, a one-way ANCOVA for repeated measures showed that there were no statistically significant covariate effects of respiratory movements on ERP data from any of the four clusters (621 ms, 633 ms, 926 ms, left and right hemisphere) ($P > 0.05$). Therefore, it is unlikely that differences in respiratory movements affected odour-related ERP changes.

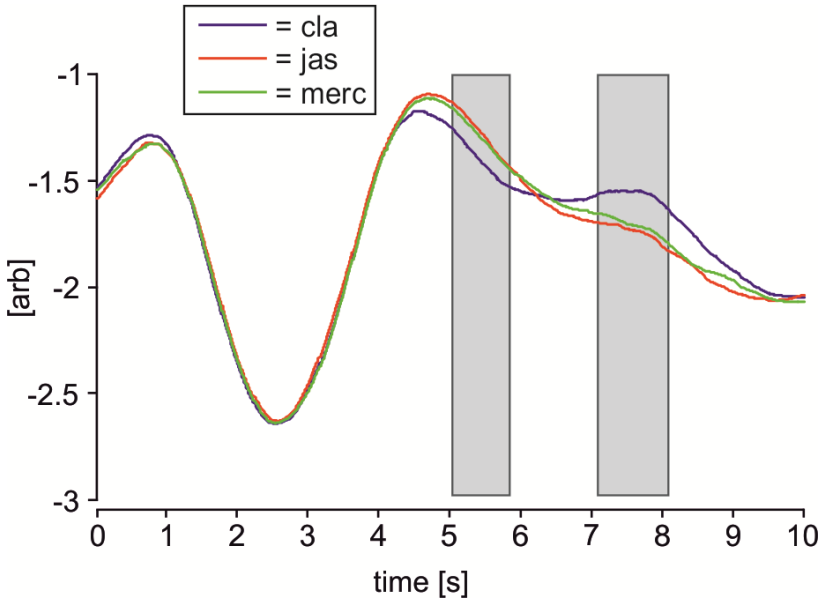

Figure 3.4: Average respiratory waveforms for each odour condition. Respiratory movement signals from every subject across all trials were averaged over a period of 10 seconds, beginning 3 seconds prior to odour onset. Time 3 represents odour onset, time 7 represents onset of the visual face stimulus. The blue line represents clean air trials (denoted as 'cla'), the red line represents pleasant odour trials ('jas') and the green line represents unpleasant odour trials ('merc'). Two grey rectangles indicate time intervals in which the three respiratory movement signals differed significantly according to a one-way ANOVA for repeated measures ($P < 0.05$). Upwards deflection of respiratory signals corresponds to inspiration.

3.5 Discussion

Our study was the first to investigate effects of pleasant and unpleasant odours on evaluations of neutral male and female faces using a novel approach to ERP analysis. We analysed ERP data from all electrodes across all time points relative to onset of the faces, to begin to provide an understanding of the processes that might underlie odour-related evaluative shifts during face perception. Behavioural data revealed the predicted effects of odours on face ratings: Neutral faces preceded by a pleasant odour prime were rated as most pleasant, whereas those preceded by an unpleasant odour prime were rated as least pleasant. Faces presented in the clean air control condition were rated in between the two. ERP data revealed that odours modulated amplitudes of late and ultra-late event-related potential components from 600 to 950 ms. Topographic maps showed greater negativity in the right posterior- and temporal-parietal electrodes in response to faces in the pleasant odour condition in clusters at 621 ms and 633 ms. At 926 ms, topographies indicated greater positivity in response to faces in the pleasant and unpleasant odour conditions in the left and right lateral fronto-temporal electrodes, respectively.

The behavioural data are consistent with previous findings that odours shift hedonic evaluations of faces (Dematte et al., 2007; Herrmann et al., 2000; Leppanen & Hietanen, 2003; Li et al., 2007; McGlone et al., 2013; Seubert et al., 2014; Todrank et al., 1995). The inclusion of a one-second interval between odour offset and face onset was also important in the present study. Our results suggest that shifts in face-evaluations were genuine priming effects evoked by the valence of the odours that carried over to the face evaluation phase, as opposed to affective responses to odours themselves.

Changes in ERP response to faces that occurred as a function of odour condition transpired during the late (> 600 ms) and ultra-late (> 900 ms) latency epochs. Indeed, the late positive potential (LPP) is known to be sensitive to pleasant and unpleasant stimuli (Cacioppo et al., 1993; Cuthbert et al., 2000; Duval et al., 2013; Hajcak et al., 2007; Hajcak et al., 2006; Weinberg & Hajcak, 2010). In evaluative priming studies, the LPP component has typically been implicated in congruency effects (Herring et al., 2011). In one ERP study using pleasant odours and faces with pleasant and unpleasant expressions, Bensafi, Pierson, et al. (2002)

showed increased LPP amplitude for unpleasant faces preceded by pleasant odour primes, presumably due to the evaluative incongruence between the two. These findings provided initial evidence that the LPP reflects evaluative processes in a cross-modal sense, where olfactory stimuli influence processing of visual stimuli. Our results provide further evidence that cross-modal effects of odours on evaluations of faces may be reflected in late ERP components.

Significant changes in late ERP components observed in the present study included increased negativity in the right posterior- and temporal-parietal electrodes in the pleasant odour condition at 621 ms and 633 ms after face onset. This latency window corresponds with the mid-LPP observed in a recent study investigating ERPs in response to faces, where the authors suggested that this component is sensitive to the emotional content of faces (Duval et al., 2013). Since our study was the first to investigate effects of briefly presented pleasant and unpleasant odour-primes on ERPs in response to faces, the present findings are novel. However, Aguado, Dieguez-Risco, Mendez-Bertolo, Pozo, and Hinojosa (2013) showed that positive targets elicited enhanced amplitudes relative to negative targets at parietal-occipital, fronto-central, and left temporal regions during the LPP. Further, Herrmann et al. (2000) showed that appetitive conditioning with a pleasant odour elicited a stronger LPP (400−600 ms) relative to a no odour control. Taken together, these findings suggest that effects of positively-valenced stimuli may take precedence during late potential components, resulting in increased ERP amplitude. In the case of the present study, effects of the pleasant odour appeared to take hold during the late potential period, increasing ERP amplitude and corresponding with increased hedonic ratings of neutral faces. The larger LPP for faces preceded by pleasant odours may reflect the general influence of pleasant odours on evaluations of neutral stimuli. This furthers our understanding of the processes underlying odour-related evaluative shifts, and may have wider implications for understanding the neural basis of pleasant odour and cleanliness perception in both evolutionary and commercial contexts.

Significant effects of odour were also observed at 926 ms after face-onset, corresponding with the late-LPP observed in another study that investigated ERP response to faces (Duval et al., 2013). Results showed increased activation over lateral frontal-temporal electrodes in response to faces presented after a

pleasant/unpleasant odour prime, in the left/right hemispheres, respectively. These findings support existing theories associating left hemisphere activity with processing of pleasant sensory stimuli, and right hemisphere activity with processing of unpleasant sensory stimuli (Ahern & Schwartz, 1985; Canli, Desmond, Zhao, Glover, & Gabrieli, 1998; Davidson, 1998; Lane et al., 1997; Lang et al., 1998; Mandal, Tandon, & Asthana, 1991; Tucker, 1981). Hemispheric specialization of positive and negative affect has rarely been investigated in the field of olfaction specifically. However, the current finding corresponds with data showing that smelling pleasant and unpleasant odours increased activation in the left and right hemispheres, respectively (Henkin & Levy, 2001). The results also lend support for the suggestion that the right hemisphere is more efficient in decoding unpleasant affects induced by odours (Bensafi, Rouby, Farget, Bertrand, et al., 2002), providing evidence that lateralization of valence processing applies to odours as well.

There was one significant and one marginally significant correlation between potential-amplitude data and baseline pleasantness ratings (taken before and after the task) at 926 ms. These suggested that participants who rated methylmercaptan as most unpleasant at baseline, showed greater activation in the left hemisphere during the late component in response to faces presented under that odour condition. Participants who perceived jasmine as more intense at baseline showed greater positive activation in the left hemisphere during the late component. However, correlations occurred with baseline ratings and during a long-latency component where there may have been a significant amount of variance. Therefore, interpretation of such correlations should be treated with caution. The lack of correlation between amplitudes and odour and face ratings suggests that strength of potentials may not precisely relate to odour-induced changes in hedonic ratings. Rather, a more general mechanism might be responsible for such effects.

One of the limitations of the present study was that, owing to a comparatively small number of face stimuli in each odour condition, effects of habituation on odour-induced changes in hedonic evaluation of faces remained unexplored. This effect was likely in the present study, as the interaction effects between experimental block and odours on face pleasantness ratings, and effect of block on odour intensity ratings pointed to a gradual decrease of hedonic effects of odours, especially in the unpleasant odour condition. Future studies involving single-trial analysis of ERPs,

and incorporating time as an independent variable in statistical analysis should address this issue.

Another issue that remained unexplored in the present study was that of potential carry-over effects of odours from one trial to the next. Effects of any residual odour carrying over into the next trial were unlikely, given the long inter-stimulus intervals and constant flow of clean air flushing out the odour tubes. However, previous research showed that the pleasantness of odours was influenced by the pleasantness of a preceding odour, as indicated by binary choices (Rolls et al., 2010) and subjective ratings (Grabenhorst & Rolls, 2009). In particular, pleasant odours preceded by a less pleasant odour were rated as more pleasant, and unpleasant odours preceded by a pleasant odour were rated as less pleasant. Such studies showed that the absolute and relative value of odours was represented in separate brain regions. In the present study, the priming effect of an unpleasant odour may have been greater in a trial preceded by a pleasant odour trial, due to the relative unpleasantness of the unpleasant odour compared to the preceding pleasant odour. Single-trial analysis of the present data could be employed to investigate whether the effects of odours on subjective ratings of faces or face-ERPs were modulated by the odour in the preceding trial. However, such analysis was beyond the scope of the present study, and these effects are unlikely given the long intervals between odour presentations.

The validity of different types of subjective rating scales; including category scales, line scales and magnitude estimation has been heavily debated, each having their advantages and disadvantages (Lawless & Malone, 1986; Lim, 2011). The vertical scaling from 0 to 100 on the scales used in the present study was an appropriate tool to measure subtle effects of odours that very slightly modulated evaluations of faces. For instance, given that the faces were all neutral, a category scale may not have been sensitive enough to pick up the small differences in ratings of faces induced by pleasant and unpleasant odours. Indeed, the differences observed between odour conditions were relatively small, but they were statistically significant. We argue that these small, but statistically significant differences provide evidence against experimenter bias. Rather than participants drastically changing their ratings of faces according to the odour context, odours provided a very subtle manipulation of subjective face ratings.

In summary, the present study used an exploratory ERP analysis to allow for the first investigation of the neural mechanisms underlying odour-induced changes in evaluations of faces. Results showed that effects of odours on face perception were reflected in late- and ultra-late ERP components. Results suggest that effects of pleasant odours on face evaluation were specific to the late component. During the ultra-late component, effects of pleasant and unpleasant odours were distinguished in the left and right hemispheres, respectively. Further, our findings show that odours can alter hedonic evaluations of faces even when there is a slight temporal lag between presentation of odours and faces. Neutral faces presented after administration of a pleasant odour were rated significantly more pleasant than the same faces presented after administration of an unpleasant odour or clean air. It is likely that any positive or negative affect induced by previous pleasant or unpleasant odour stimulation carried over into the face evaluation phase.

Chapter 4

Effects of stimulus onset asynchrony on odour induced hedonic evaluations of faces: an event-related potential study.

This experiment investigated the effects of pleasant and unpleasant odours on evaluations of faces and face ERPs using two different stimulus onset asynchronies.

It is currently under review for European Journal of Neuroscience.

The roles of the co-authors are summarised below:

I designed the study in collaboration with Andrej Stancak and collected the data. Katerina Kokmotou, Vicente Soto, Hazel Wright and Nicholas Fallon assisted with the data collection. Andrej Stancak and Nicholas Fallon provided training during the data analysis. I analysed the data, interpreted the results, and wrote the manuscript. Katerina Kokmotou, Vicente Soto, Hazel Wright, Nicholas Fallon, Anna Thomas, Timo Giesbrecht, Matt Field and Andrej Stancak all provided useful comments whilst preparing the manuscript for publication.

4.1 Abstract

Odours alter evaluations of concurrently presented visual stimuli, such as faces. Stimulus onset asynchrony (SOA) refers to the temporal association between prime and target stimuli, and is known to affect evaluative priming in various sensory modalities. However, effects of SOA on odour priming of visual stimuli are not known. The present study aimed to analyse whether subjective and cortical activation changes during odour priming would vary as a function of SOA between odours and faces.

Twenty-eight participants rated faces under pleasant, unpleasant, and no-odour conditions using visual analogue scales. In half of trials, faces appeared one-second after odour offset (SOA 1). In the other half of trials, faces appeared during the odour pulse (SOA 2). EEG was recorded continuously using a 128-channel system, and event-related potentials (ERPs) to face stimuli were evaluated using statistical parametric mapping.

Faces presented during unpleasant-odour stimulation were rated significantly less pleasant than the same faces presented one-second after offset of the unpleasant odour. Activation clusters in the late-positive-potential (LPP) were stronger for faces presented simultaneously with the unpleasant odour compared to the same faces presented after odour offset. Face pictures presented after an unpleasant odour were also associated with changes in the respiratory pattern, and these changes were related to the cortical activation changes in the LPP period.

A greater cortical and subjective response during simultaneous presentation of faces and unpleasant odour may have an adaptive role, allowing for a prompt and focused behavioural reaction to a concurrent stimulus if an aversive odour would signal danger.

4.2 Introduction

Previous studies have shown that pleasant and unpleasant odours influence evaluations of human faces (Bensafi, Pierson, et al., 2002; Cook et al., 2015; Dematte et al., 2007; Leppanen & Hietanen, 2003; Li et al., 2007; McGlone et al., 2013; Seubert et al., 2014; Todrank et al., 1995). However, the neural mechanisms that underlie such effects are not well established. Functional magnetic resonance imaging (fMRI) data suggested that faces paired with pleasant fragrance activated the medial orbitofrontal cortex, implicated in encoding the reward value of stimuli; whilst faces paired with unpleasant odour activated the amygdala, known to be involved in the processing of aversive stimuli (McGlone et al., 2013). ERP data revealed that late ERPs evoked by faces were modulated by the presence of pleasant and unpleasant odours (Bensafi, Pierson, et al., 2002; Cook et al., 2015).

The strength of odour priming, manifesting in changes of hedonic evaluations of concurrently presented visual stimuli and in associated brain activation patterns, is likely affected by the temporal association between the prime (odour) and the target (visual stimulus), known as stimulus onset asynchrony. Studies investigating affective priming using words and pictures suggest that the stimulus onset asynchrony between prime and target stimuli is of importance (De Houwer, 1998; D. D. H. Hermans, J. Eelen, P., 2001). A recent meta-analysis of evaluative priming pointed to SOA as a factor influencing the strength of priming across verbal and non-verbal stimuli (Herring et al., 2013). The authors showed that SOA effects manifest in a decreased change of hedonic evaluation of targets with long, compared to short intervals between the prime and target.

The effects of SOA on odour priming are not known. Most previous studies investigating effects of odours on immediate evaluations of faces used paradigms where the odour primes and target faces overlapped (Dematte et al., 2007; Leppanen & Hietanen, 2003; Seubert et al., 2014), or where target faces appeared at the offset of the odour prime (Bensafi, Pierson, et al., 2002), or one second after offset (Cook et al., 2015; Seubert et al., 2010). However, it is not known whether there are differences in effects of odours on hedonic evaluations of faces, either behaviourally

or reflected in ERPs, when faces are presented during odour stimulation compared to when they are presented after odour offset.

The aim of the present study was to investigate the effects of pleasant and unpleasant odours on evaluations of neutral male and female faces presented during odour stimulation and after odour offset. In line with previous findings of SOA effects on the strength of evaluative priming (Herring et al., 2013), we hypothesised that odour-induced changes in evaluations of faces and the long-latency components of ERPs would be stronger when faces appeared during the odour pulse compared to when they were presented one second after odour offset.

4.3 Methods and Materials

4.3.1 Participants

A total of 29 (10 male) participants aged 18−31 years (23.6 ± 3.8, mean ± standard deviation) took part in the experiment after responding to an advertisement. All but four participants were right-handed. One participant withdrew from the experiment. EEG data from two participants were subsequently excluded due to excessive amounts of artifacts. Hence, behavioural data from 28 subjects, and EEG data from 26 (10 male) subjects were used in the analysis. People suffering from asthma or neurological disorders, particularly anosmia or epilepsy, were not permitted to take part in the study. Normal olfactory function was ascertained using the Sniffin'Sticks (Hummel et al., 1997) test battery. Participants had to successfully identify a minimum of 9 out of the 12 odours in order to take part in the experiment. Participants were asked not to smoke, drink coffee or chew gum for two hours prior to the experiment, and were asked to minimise their use of fragranced products on the day. Participants were reimbursed for their time and travel expenses. The study was approved by the Research Ethics Committee at the University of Liverpool. All participants gave written informed consent in accordance with the Declaration of Helsinki.

4.3.2 Visual and olfactory stimuli

A total of 90 (45 male) neutral faces were used in the experiment. Due to the large number of faces needed to satisfy the number of trials required per condition, faces were selected from three databases. Forty-two (24 male) faces were obtained from the NimStim Set of Facial Expressions (Tottenham et al., 2009). Forty-three (21 male) faces were obtained from the Japanese and Caucasian Neutral Faces (JACNeuf; Matsumoto & Ekman, 1988). A further five female faces were selected from the Gur/Kohler images, acquired according to Gur et al. (2002) and referenced in Kohler et al. (2003). All face images were frontal views, in colour, with a consistent light background and similar dimensions. During the screening session, participants rated the perceived pleasantness of the facial expressions of all 90 faces (on a scale ranging from 0 – very unpleasant to 100 – very pleasant) in order to ensure that they were perceived as neutral. The mean face pleasantness rating was 50.3 (± 8.4).

Odours were administered through two tubes approximately two centimetres away from the nostrils; using a custom-built, continuous airflow, computer-controlled olfactometer with 8 channels (Dancer Design Ltd., UK). Odour pulses were embedded within a constant flow of clean air, in order to avoid any effects of a sudden increase in airflow associated with presentation of an odour (Huart et al., 2012). Airflow was kept constant at 2.5 l/min.

There were three odour conditions in the experiment; pleasant, unpleasant and a neutral, 'clean air' control. Methylmercaptan (1% dilution in Propylene Glycol), a rotten cabbage-like odour, was selected for the unpleasant condition. Jasmine odour (no dilution) was selected for the pleasant condition. These dilutions were matched on perceived intensity based on data from a previous experiment (Mean intensity rating of Jasmine: 56.33 ± 15.83, mean intensity rating of Methylmercaptan: 61.34 ± 17.68; Cook et al., 2015). Odours were supplied by Symrise Ltd. (Netherlands). Propylene Glycol (1,2-Propanediol 99%, Sigma-Aldrich Ltd., UK) was used for dilution, the clean air control and constant flow.

Both presentation of the experimental task stimuli and triggering of the odour valves were achieved using the Cogent v. 1.32 program (Wellcome Department of

Imaging Neuroscience, United Kingdom) running in Matlab v. R2011a (The MathWorks, Inc., USA). In between experimental blocks and sessions, a Blueair 203 air purifier (Blueair Ltd., Sweden) was used to minimise any residual odour that may have carried into the next experimental block or session.

4.3.3 Recordings

EEG was recorded continuously using a 128-channel Geodesics EGI System (Electrical Geodesics, Inc., Eugene, Oregon, USA) with the sponge-based Geodesic Sensor Net. The sensor net was aligned with respect to three anatomical landmarks; two pre-auricular points and the nasion. Electrode-to-skin impedances were kept below 50 kΩ and at equal levels across all electrodes. The recording band-pass filter was 0.01−1000 Hz, and the sampling rate was 1000 Hz. The electrode Cz was used as the reference.

Participants' respiratory movements and pulse pressure were recorded continuously throughout the experiment with a piezoelectric respiratory belt transducer worn around the chest at the level of the epigastrium, and a finger pulse oximeter transducer worn on the index finger of the left hand (ADInstruments Ltd., Oxford, UK). Signals were transduced and extracted using LabChart 7 (ADInstruments Ltd., Oxford, UK).

4.3.4 Procedure

After application of the EEG net, participants were seated in a dimly lit, sound attenuated room facing a 19 inch LCD monitor (60 Hz refresh rate) placed approximately 0.7 m in front of them. First, the respiratory belt and pulse pressure sensor were fitted onto participants and the signals were checked. Following this, the olfactometer head piece was fitted, and participants were given instructions. The experimental session lasted around 1.5 hours in total, including baseline odour ratings and the experimental task. Ratings of odour pleasantness, intensity, and familiarity were recorded before and after the task. Each odour was administered

individually, in a four-second pulse manually triggered to coincide with the onset of inspiration. After each odour pulse, on-screen visual analogue scales prompted participants to rate the pleasantness (from 0 – very unpleasant to 100 – very pleasant), intensity (0 – no odour to 100 – very intense odour) and familiarity (0 – not familiar at all to 100 – extremely familiar) of the odour.

The experimental task was split into four blocks of 45 trials (180 trials in total). Trials were pseudo-randomly ordered such that each of the 90 faces used in the task appeared twice: once under each SOA condition, with the same odour both times. Any given face never appeared more than once in one block. Odour presentation was also pseudo-random, such that all three odours were presented across all four blocks, but no two consecutive trials used the same odour. Figure 4.1 shows a flowchart of the trial procedure. Each trial began with a resting interval during which participants viewed a white cross on a black background. The duration of this interval was dependent upon the triggering of the odour pulse; the experimenter observed participants' respiratory waveforms, and manually triggered the odour pulses at the very onset of inspiration. In half of the trials, a three-second odour pulse was released, during which time participants viewed a black screen. The screen remained black for a further one-second resting interval after odour offset, before a neutral face was displayed on-screen for 300 ms (SOA 1). The other half of the trials were identical, apart from that the neutral face was displayed on-screen during the three-second odour pulse, at 2000 ms after odour onset (SOA 2). In both conditions, a resting interval with a black screen then preceded a rating scale prompting participants to rate the pleasantness of the neutral face (from 0 – very unpleasant to 100 – very pleasant). Once participants had responded, a second scale prompted them to rate the intensity of the odour administered in that trial (0 – no odour to 100 – very intense odour). After their response, the next trial began.

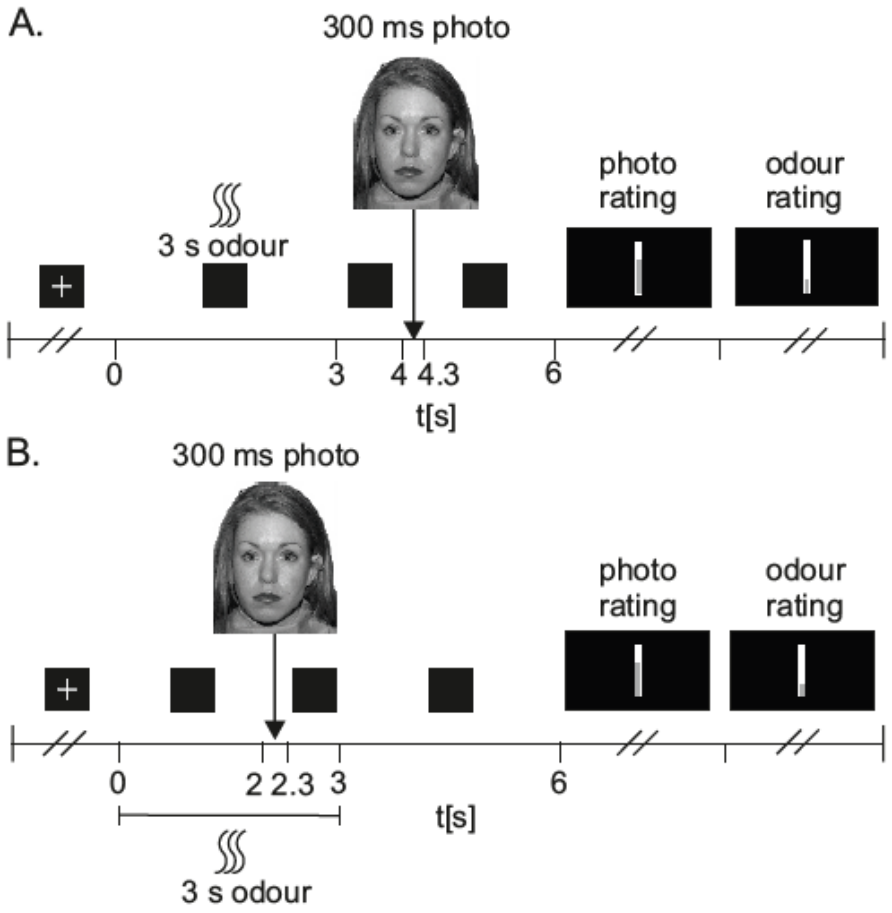

Figure 4.1: Flowchart of experimental trial procedure.

4.3.5 Behavioural analysis

Ratings of odour pleasantness, intensity and familiarity taken before and after the experimental task were collapsed and analysed using paired t-tests. Data from the experimental task were analysed using 2 × 3 repeated measures ANOVAs, observing differences in face pleasantness ratings and odour intensity ratings with odour condition (pleasant, unpleasant, and neutral) and SOA as the independent variables. Significant main effects were investigated using pairwise comparisons; significant interactions were followed up with post-hoc t-tests, using Bonferroni correction for multiple comparisons. P values in all ANOVA effects were adjusted using the

Greenhouse-Geisser ε method. All behavioural data was analysed using SPSS v. 22 software package (IBM Inc., USA).

4.3.6 ERP analysis

EEG recordings were pre-processed using BESA v. 6.0 (MEGIS GmbH, Germany). Data were first referenced to a common average using the common averaging method (Lehmann, 1987). The oculographic and, when necessary, electrocardiographic artifacts were removed by principal component analysis (Berg and Scherg, 1994). Data were visually inspected for the presence of any movement or muscle artifacts, and trials contaminated with artifacts were excluded. The mean number of accepted trials across all subjects and all conditions was 160 (SD = 17.5). Participants were excluded from the analysis if the number of trials accepted was less than 125 (2 standard deviations from the mean).

Data were band-pass filtered from 2−35 Hz and down-sampled to a rate of 256 Hz, and exported from BESA into the SPM12 software package (Statistical Parametric Mapping, UCL, England; http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Event-related potentials (ERPs) in response to neutral faces were computed separately for each odour and SOA condition by averaging respective epochs in the intervals ranging from 300 ms before photo onset to 1000 ms after photo onset. The baseline period ranged from -300 ms to 0 ms relative to the onset of the visual stimulus.

We applied an omnibus analysis of the effects of odours on ERPs involving all time points from 0 ms to 1000 ms and all scalp sites, allowing us to explore the effects of odours on ERPs without applying a priori knowledge of peak latencies. The SPM12 toolbox combines advanced statistical models with robust control for Type I error (Poline et al., 1997; Kiebel & Friston, 2004). In contrast to alternative approaches, such as permutation analysis of clusters of ERPs over the epoch time (Maris & Oostenveld, 2007), SPM applies the theory of random fields to volumes of space-time data. This allows for calculation of the degrees of freedom in the evaluation of statistical test results based on the spatial and temporal complexity of data (Worsley, 2003).

The statistical analysis was performed in two steps. In the initial exploratory step, EEG data were converted into three-dimensional scalp-time images using SPM. The electrodes were mapped onto a standardised scalp grid sized 32 × 32 pixels (pixel size 4.25 × 5.3 mm^2), representing the field potential planes stacked over the time axis. Images were smoothed with a Gaussian kernel of 9 × 9 × 20 mm^2 .ms (full width at half maximum). Data from over the whole epoch (385 time samples) and all standardised scalp points were screened for statistically significant effects of odours and SOA using a flexible factorial ANOVA for repeated measures. The flexible factorial model in SPM allows for the inclusion of the subject factor as an independent variable. We applied an uncorrected threshold of $P < 0.001$, and a cluster size threshold of 20 contiguous space-time voxels to detect clusters affected by odours and SOA. The amplitude data from these clusters were subsequently analysed using further repeated measures ANOVAs in SPSS v. 22 (IBM Inc., USA). The statistical threshold of this confirmatory analysis was $P < 0.05$.

4.3.7 Analysis of respiratory movements

Respiratory movement signals were low-pass filtered, and averaged separately for each of the six conditions in the epoch of interest, then analysed statistically using a 2 × 3 repeated measures ANOVA (2 SOAs, 3 odours). The 7 s analysis epoch ranged from odour onset (t = 0 s) to 7 s after odour onset. Therefore, the intervals 2–3 s and 4–5 s coincided with the ERP analysis epoch for SOA 2 and SOA 1, respectively. To control for Type I error due to the large number of ANOVAs given that one ANOVA was computed on each time sample, a permutation analysis with 500 permutations was used to correct the P values (Maris & Oostenveld, 2007). Data from the interval showing a significant effect of condition on respiratory movements were analysed using a confirmatory repeated measures ANOVA in SPSS. We used a 2 × 3 ANCOVA for repeated measures in BMDP 2V program (Biomedical Data Package, Cork, Ireland) to analyse whether changes in respiratory movement patterns contributed to the effects of experimental condition observed in ERP clusters.

4.4 Results

4.4.1 Odour ratings

Mean ratings of odour pleasantness, intensity and familiarity taken before and after the experimental task were collated and are shown in Table 4.1. Paired t-tests confirmed that jasmine was rated as significantly more pleasant than methylmercaptan ($t(27) = 28.34$, $P < 0.001$); there was no significant difference in intensity ratings of jasmine and methylmercaptan ($t(27) = -1.64$, $P = 0.11$), and there was no significant difference in familiarity ratings of jasmine and methylmercaptan ($t(27) = 0.72$, $P = 0.48$).

Table 4.1: Mean (± standard deviation) ratings of odour pleasantness, intensity and familiarity that were taken before and after the experimental task and concatenated.

Odour	Pleasantness	Intensity	Familiarity
Jasmine	78.17 (± 11.15)	75.56 (± 10.86)	68.36 (± 20.53)
Methylmercaptan	13.0 (± 10.38)	80.09 (± 9.69)	65.71 (± 24.45)

4.4.2 Face and odour ratings

Table 4.2 shows the mean ratings of faces under each odour and SOA condition. A repeated-measures ANOVA revealed a significant main effect of odour on ratings of faces ($F(2, 54) = 14.63$, $\eta_p^2 = 0.35$, $P < 0.001$). Pairwise comparisons indicated that faces in the unpleasant odour condition were rated as significantly less pleasant in comparison to faces in both the control ($P < 0.001$) and pleasant odour ($P = 0.001$) conditions. There was no significant difference between ratings of faces in the control and pleasant odour conditions ($P > 0.05$). There was no main effect of SOA on ratings of faces ($F(1, 27) = 0.23$, $\eta_p^2 = 0.01$, $P = 0.64$). However, there was a significant interaction between odours and SOA affecting face ratings ($F(2, 54) = 3.3$ $\eta_p^2 = 0.11$, $P = 0.05$). Further analysis of this interaction (using a 2 × 2 repeated

measures ANOVA) showed that the effect was caused by the greater pleasantness of faces presented during averaged clean air and pleasant odour conditions and greater unpleasantness of faces presented during the unpleasant odour condition in SOA 2 compared to SOA 1 ($F(1, 27) = 5.02$, $P = 0.034$). The interaction appeared to be driven by the contrast between SOAs in the unpleasant odour condition ($F(1, 27) = 3.29$, $P = 0.081$), since this contrast was comparatively weak in the pleasant odour and clean air conditions ($F(1, 27) = 1.07$, $P = 0.31$).

Table 4.2: Mean (± standard deviation) ratings of neutral faces under each odour and SOA condition (SOA 1 – faces presented one second after odour offset, SOA 2 – faces presented during odour stimulation).

SOA	Clean Air	Jasmine	Methylmercaptan
SOA 1	53.76 (± 6.23)	53.30 (± 7.57)	49.39 (± 5.75)
SOA 2	54.11 (± 7.10)	53.81 (± 7.81)	47.94 (± 7.58)

Table 4.3 shows the mean odour intensity ratings acquired from experimental trials for each odour and SOA condition. A repeated-measures ANOVA revealed a significant main effect of odour on intensity ratings ($F(2, 54) = 209.6$, $\eta_p^2 = 0.89$, $P < 0.001$). Pairwise comparisons indicated that both the pleasant and unpleasant odour were rated as significantly more intense than clean air ($P < 0.001$). There was no significant difference between intensity ratings of the pleasant and unpleasant odours ($P > 0.05$). There was a significant main effect of SOA affecting odour intensity ratings ($F(1, 27) = 6.97$, $\eta_p^2 = 0.21$, $P = 0.01$), suggesting that odours were rated more intense on trials using SOA 2 (40.79 ± 28.84) in comparison to trials using SOA 1 (39.37 ± 28.71). There was no significant interaction between odour and SOA condition affecting odour intensity ratings during the experiment ($P > 0.05$).

Table 4.3: Mean (± standard deviation) odour intensity ratings acquired from experimental trials for each odour and SOA condition.

SOA	Clean Air	Jasmine	Methylmercaptan
SOA 1	4.33 (± 4.0)	54.82 (± 16.21)	58.95 (± 18.35)
SOA 2	4.71 (± 4.35)	57.05 (± 14.35)	60.59 (± 17.36)

4.4.3 ERP components

Figure 4.2 illustrates the event-related potentials in response to faces across all trials and all odour conditions in the form of a butterfly plot and topographic maps of selected potential components. Topography of the first component showed bilateral positivity over the occipital electrodes and negativity over frontal electrodes, peaking at around 100 ms. This is consistent with characteristics of the P1 component, which is related to early processing of visual stimuli (Hopf et al., 2002). The second component, peaking around 205 ms, showed negativity over parietal and temporal electrodes, consistent with characteristics of the N170 face-processing component (Bentin et al., 1996). The next component peaked around 430 ms, showing strong negativity over occipital and parietal electrodes, consistent with the N400 component, which is implicated in the processing of meaningful stimuli, including faces (Kutas & Federmeier, 2011). The final component was a long component beginning around 500 ms and peaking at approximately 530 ms. Showing negativity over occipital electrodes sites and positivity over central areas, it was consistent with characteristics of the late positive potential (LPP), which is sensitive to the emotional content of pictures, words and faces (Cacioppo et al., 1993; Cuthbert et al., 2000; Hajcak et al., 2007; Hajcak et al., 2006).

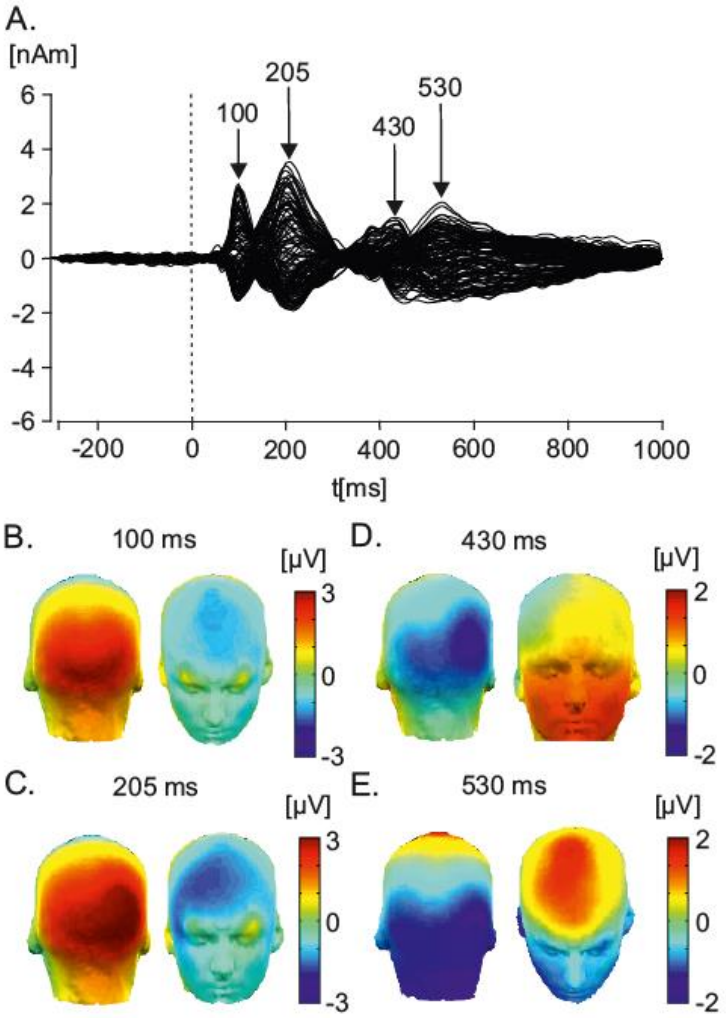

Figure 4.2: Butterfly plot of grand average ERP responses to faces and corresponding scalp topographies. (A) Butterfly plot of grand average ERPs in response to faces. Peak latencies of distinct ERP components (100 ms, 205 ms, 430 ms, and 530 ms) are highlighted with arrows. (B) Latency component 100 ms (P1). The topographic maps of grand average ERPs overlaid on the volume rendering of the human head are shown. (C) Latency component 205 ms (N170). (D) Latency component 430 ms (N400). (E) Latency component 530 ms (late component/LPP).

4.4.4 Effects of odours and SOA on ERPs

SPM12 was used to compute a 2 × 3 repeated measures ANOVA on smoothed scalp-time images of data from 0−1000 ms relative to the onset of the face. The ANOVA revealed scalp-time clusters showing significant main and interaction effects of SOA and odour on the ERP responses to faces. Figure 4.3 illustrates these significant scalp-time clusters. The corresponding topographic maps from each

odour/SOA condition for each significant cluster are shown with bar graphs representing the mean EEG scalp-amplitude (μV).

There was a main effect of SOA on ERP responses to faces at 169 ms and 173 ms after onset of the face (uncorrected $P < 0.001$), during the N170 component (see Figure 4.3A). Given that the two clusters were within 20 ms of one another, it is likely that they reflect the same process. Further, the cluster at 169 ms showed positive amplitude, whilst the cluster at 173 ms showed negative amplitude; it is therefore reasonable to assume that these clusters formed a dipole. Subsequent t-tests performed on EEG amplitude data from these two clusters showed that faces presented using SOA 2 yielded stronger EEG amplitude at both the 169 ms cluster ($t(25) = -5.49$, $P < 0.001$), and the 173 ms cluster ($t(25) = 5.67$, $P < 0.001$) compared to faces presented using SOA 1.

Another statistically significant scalp-time cluster represented a main effect of odour on the ERP response to faces at 391 ms following onset of the face (uncorrected $P < 0.001$), in the left frontal electrodes during the N400 component (see Figure 4.3B). A confirmatory one-way ANOVA in this cluster showed a significant effect of odour ($F(2, 50) = 16.33$, $\eta_p^2 = 0.4$, $P < 0.001$). Pairwise comparisons indicated that there were significant differences in EEG amplitude between all three odour conditions ($P < 0.05$): faces in the clean air condition produced the lowest amplitude (0.33 ± 1.14), and faces in the unpleasant odour condition produced the highest amplitude (1.09 ± 0.98). Faces in the pleasant odour condition produced an amplitude between the two (0.68 ± 1.09).

An interaction between odour and SOA yielded a significant effect (uncorrected $P < 0.001$) on the ERP response to faces in scalp-time clusters at 516 ms ($F(2, 50) = 9.81$ $\eta_p^2 = 0.28$, $P = 0.001$) and 712 ms ($F(2, 50) = 12.81$, $\eta_p^2 = 0.34$, $P < 0.001$) following onset of the face. Post-hoc t-tests revealed significantly greater amplitudes of positive and negative potential components for faces presented in the unpleasant odour condition using SOA 2, in the 516 ms cluster (1.00 ± 0.60, $P = 0.002$) and the 712 ms cluster ($-0.90, \pm 0.73$, $P < 0.001$), respectively. There were no significant differences in EEG amplitude between SOA 1 and SOA 2 in the clean air or pleasant odour conditions ($P > 0.05$).

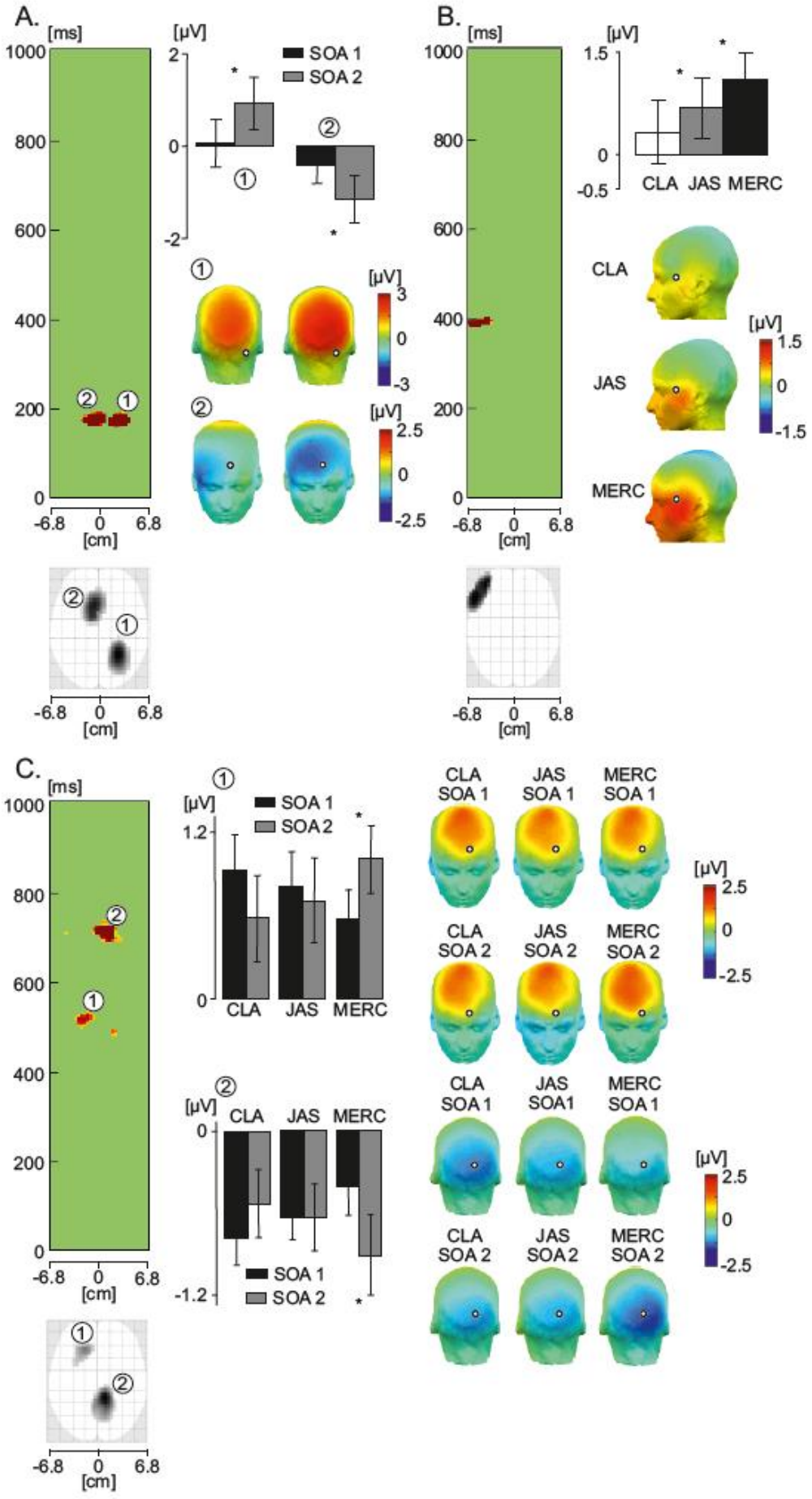
A.
[ms]
1000
800
600
400
200
0
-6.8
0
6.8
[cm]
[μV]
2
0
-2
SOA 1
SOA 2
①
②
[μV]
3
0
-3
2.5
0
-2.5
B.
1.5
-0.5
CLA
JAS
MERC
C.
1.2
CLA SOA 1
JAS SOA 1
MERC SOA 1
CLA SOA 2
JAS SOA 2
MERC SOA 2
JAS SOA1

Figure 4.3: Two-way ANOVA showing the effects of odour and SOA conditions on ERP responses to faces. (A) Main effect of SOA on ERP responses to faces across all odour conditions. The green panel shows statistically significant latency periods ($P < 0.05$ FWE) in the scalp-time plot where F values represent the strength of variance between SOA conditions over the horizontal axis of the scalp in every time sample from 0 ms and 1000 ms relative to the onset of the face photograph. The scalp values over the horizontal axis of the scalp are averages of F values occurring at each vertical point for a given horizontal point in the standardised scalp map (from -6.8 cm to +6.8 cm). There were two spatio-temporal clusters showing a statistically significant effect of SOA around the N170 component. Below the green panel is the standard scalp map of statistically significant clusters using ERPs. The first significant cluster, labelled 1, occurred at 169 ms and had positive amplitude. The second, labelled 2, occurred at 173 ms and had negative amplitude. Bar graphs illustrate the mean EEG amplitude for each cluster under each SOA condition (μV). Black bars represent SOA 1, and grey bars represent SOA 2. Asterisks indicate statistically significant differences between SOA 1 and SOA 2 ($P < 0.05$). Corresponding topographic maps of the numbered significant clusters for the two SOA conditions are shown. White circles with a black outline pinpoint the location of the significant electrode clusters. (B) Main effect of odour condition on ERP responses to faces across both SOA conditions. The green panel shows statistically significant latency periods ($P < 0.05$ FWE) in the scalp-time plot where F values represent the strength of variance between odour conditions. One spatio-temporal cluster showed a statistically significant effect of odour around the N400 component. Below the green panel is the standard scalp map of the statistically significant cluster. Bar graphs illustrate the mean EEG amplitude at this cluster under each odour condition (μV). The white bar represents the clean air condition (labelled CLA), the grey bar represents the pleasant odour condition (labelled JAS) and the black bar represents the unpleasant odour condition (labelled MERC). Asterisks indicate statistically significant differences between odour conditions ($P < 0.05$). Corresponding topographic maps of the significant cluster for the three odour conditions are shown. (C) Interaction between odour and SOA condition affecting ERP responses to faces. The green panel shows statistically significant latency periods ($P < 0.001$ uncorrected) in the scalp-time plot. Two spatio-temporal clusters during the LPP showed were significantly affected by an interaction between odour and SOA conditions. Below the green panel is the standard scalp map of the statistically significant clusters. The first significant cluster, labelled 1, occurred at 516 ms and had positive amplitude. The second, labelled 2, occurred at 712 ms and had negative amplitude. Bar graphs illustrate the mean EEG amplitude for each cluster under each condition (μV). Black bars represent SOA 1, and grey bars represent SOA 2. Odour conditions are labelled CLA, JAS, and MERC. Asterisks indicate the statistically significant differences between SOA 1 and SOA 2 in the unpleasant odour condition ($P < 0.025$, Bonferroni corrected). Corresponding topographic maps of the numbered significant clusters for all conditions are shown.

4.4.5 Respiratory movements

Figure 4.4 shows averaged respiratory waveforms for each condition in a 7 s interval, beginning at odour onset. A repeated-measures ANOVA (2 SOAs, 3 odours) showed a statistically significant interaction between odour and SOA in the interval 4805–5010 ms. This interval overlapped with the period in which ERPs were recorded and analysed for trials using SOA 1. To analyse this interaction effect further, respiratory movement data from this interval were subjected to a repeated measures ANOVA in SPSS. Post hoc t-tests revealed that this interaction was driven by a significant difference between respiratory movements in trials using SOA 1 and SOA 2 in the unpleasant odour condition only ($t(25) = 2.29$, $P = 0.03$). Upon visual inspection of each individual's respiratory waveforms, it appeared that 16 subjects

tended to inspire during the 4–5 s interval on trials in the unpleasant odour condition using SOA 1.

Repeated measures ANCOVA using the ERP cluster peaking at 712 ms as the dependent measure and the amplitude of respiratory movements as a covariate showed that there was a significant covariate effect of respiratory movements in the 712 ms ERP cluster ($F(1, 49) = 4.24$, $P = 0.05$). When this covariate effect was taken into account, the significance of the interaction between odour and SOA affecting the ERP cluster decreased very slightly ($F(2, 49) = 9.01$, $P < 0.001$), but remained statistically significant.

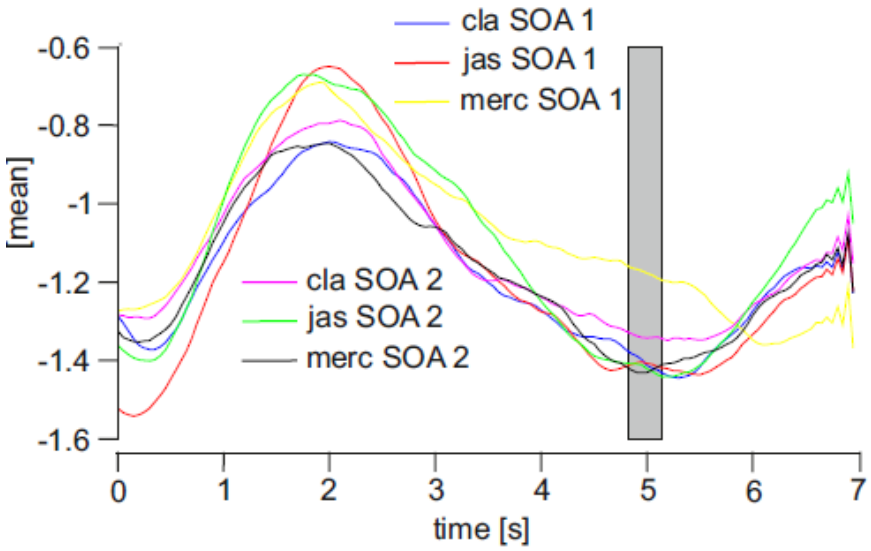

Figure 4.4: Average respiratory waveforms for each condition. Respiratory movement signals from every subject across all trials were averaged over a period of 7 seconds, beginning at odour onset (Time 0). Time 2 represents onset of the visual face stimulus in trials using SOA 2, and Time 4 represents onset of the visual face stimulus in trials using SOA 1. The blue line represents clean air trials using SOA 1 (denoted as 'cla SOA 1'), the red line represents pleasant odour trials using SOA 1 ('jas SOA 1') and the yellow line represents unpleasant odour trials using SOA 1 ('merc SOA 1'). The pink line represents clean air trials using SOA 2 ('cla SOA 2'), the green line represents pleasant odour trials using SOA 2 ('jas SOA 2') and the black line represents unpleasant odour trials using SOA 2 ('merc SOA 2') The grey rectangle indicates time intervals where respiratory movement signals differed significantly according to a two-way ANOVA for repeated measures ($P < 0.05$). Upwards deflections of respiratory signals correspond to inspiration.

4.5 Discussion

Effects of SOA on odour-related priming of faces manifested in hedonic evaluations, cortical potentials, and respiratory activity. In particular, unpleasant odours had a greater effect on hedonic evaluations and cortical responses when faces were presented during odour stimulation.

In accordance with previous studies (Cook et al., 2015; Li et al., 2007; Todrank et al., 1995), neutral faces presented with or after unpleasant odour stimulation were rated as significantly less pleasant than faces in both the control and pleasant odour conditions. These odour priming effects occurred with and without a temporal lag between odour and face presentation, suggesting that odours have the capacity to alter hedonic evaluations of visual stimuli, even if they are presented shortly after offset of the odour.

In line with previous studies (Bensafi, Pierson, et al., 2002; Cook et al., 2015), the results also showed that odours affected the amplitudes of ERP components. In particular, odours affected the N400 component. The N400 component is typically associated with semantics and language processing, but is known to be involved in processing the contextual information about stimuli, including faces (Kutas & Federmeier, 2011). Our data showed that faces in the unpleasant odour condition produced the largest N400 amplitude, whilst faces in the clean air condition produced the smallest N400 amplitude. Faces in the pleasant odour condition produced amplitude that was between the two. In this case, the N400 may have represented contextual information induced by the odours, with the unpleasant odour being the most salient context, the pleasant odour being the next most salient, and clean air a neutral context. This further supports data showing that negative stimuli influence evaluations more strongly than positive stimuli of comparable intensities (T. A. Ito, Larsen, Smith, & Cacioppo, 1998; Smith, Cacioppo, Larsen, & Chartrand, 2003; Smith et al., 2006). The significant differences in the N400 response to faces between all odour conditions suggest that whilst the behavioural task may not have been sensitive enough to pick up differences in evaluations of faces between the pleasant odour and control conditions, the N400 was able to differentiate the context in which faces were presented.

The key finding referred to an interaction between odour condition and SOA where faces presented during clean air and pleasant odour conditions were rated as more pleasant, and faces presented during unpleasant odour stimulation were rated as less pleasant, than the same faces presented one second after odour offset. This interaction appeared to be driven by the contrast effect of SOA in the unpleasant odour condition, an effect that was mirrored in the EEG data during the LPP. Our data showed that faces presented during unpleasant odour stimulation were associated with greater LPP amplitude than the same faces presented one second after offset of the unpleasant odour. The LPP is known to be sensitive to the valence of pictures, words and faces (Cacioppo et al., 1993; Cuthbert et al., 2000; Hajcak et al., 2007; Hajcak et al., 2006). Previous research has suggested that the LPP responds to the emotional content of faces (Duval et al., 2013), and that contextual information integrates with face processing during the LPP (Dieguez-Risco, Aguado, Albert, & Hinojosa, 2013). The present results further suggest that the LPP may indeed be sensitive to the emotional content of faces, or likely the emotional context in which they are presented. Stronger ERP amplitudes and more significant changes in hedonic ratings during simultaneous unpleasant odour and neutral face stimulation support the evidence for an attentional bias towards negative stimuli (T. A. Ito et al., 1998; Smith et al., 2003; Smith et al., 2006), and are consistent with previous findings that negative odours elicit faster reactions than other odours (Boesveldt et al., 2010). Taken together, the results suggest that odour priming effects were stronger during simultaneous odour and face presentation, at least in the unpleasant odour condition, and that the LPP may represent the effects of unpleasant odour context on face perception. A greater cortical and subjective response during simultaneous olfactory and visual stimulation may have an adaptive role, allowing for a prompt and focused behavioural reaction if an aversive odour would signal danger.

A parallel interaction between odours and SOA to that seen in the LPP components was observed in the respiratory movement data: there was a significant difference between respiratory movements in trials using SOA 1 and SOA 2 in the unpleasant odour condition only. There was a significant covariate effect of respiratory movement data on an LPP (712 ms) scalp-time cluster, however, the interaction between SOA and odour remained significant when this covariate effect

was taken into account. Odour perception and odour induced emotions are dependent upon inspiration (Homma & Masaoka, 2008), and given that the amygdala and entorhinal cortex receive direct inputs from the olfactory bulb and piriform cortex (McDonald, 1998), it is not surprising to find that odour priming also affects respiratory movements. We observed that participants tended to inspire during presentation of face photographs presented one second after the offset of an unpleasant odour. Previous studies have shown differences in respiratory patterns during presentation of high arousal stimuli (Gomez, Stahel, & Danuser, 2004; Gomez, Zimmermann, Guttormsen-Schär, & Danuser, 2005; Ritz, George, & Dahme, 2000), and a number of studies have found increases in respiratory activity during induction of negative emotional states (see review by Boiten, Frijda, & Wientjes, 1994). Indeed, one study confirmed that unpleasant odours increase respiratory rate and induce rapid shallow breathing (Masaoka, Koiwa, & Homma, 2005). Authors have argued that the valence and arousal effects may reflect energy mobilisation in preparation to act, and a manifestation of attentional bias towards negative stimuli (Gomez et al., 2004). In the present study, the unpleasant odour may have increased arousal, resulting in increased inspiration, analogous with the notion of attentional bias and preparation for a behavioural reaction in the presence of aversive stimuli. However, given that this effect was only observed when faces were presented one second after the unpleasant odour, such interpretation should be treated with caution.

SOA also affected the early face ERP component (N170) independently of the type of odour. The N170 component was stronger when faces were presented during odour stimulation in comparison to those same faces presented one second after odour offset. This finding is consistent with the suggestion that odours can influence early stages of visual processing (Robinson, Reinhard, & Mattingley, 2014), and the finding that the N170 is modulated by emotional context (Righart & de Gelder, 2006). Indeed, one recent study showed enhancement of the EEG response between 130 and 180 ms after face onset when faces were presented with an odour (Leleu, Godard, et al., 2015). It is likely that the multisensory stimulation experienced when odours and faces were presented together resulted in increased allocation of attentional resources and increased N170 amplitude as a consequence. This explanation is supported by increased perceived odour intensity observed in

trials when odours were presented simultaneously with faces. It is possible that odour context boosts face processing and corresponding ERPs, and that simultaneous presentation of odour and face stimuli results in increased odour intensity perception.

Whilst the unpleasant odour appeared to reduce hedonic ratings of neutral faces, there were no differences between ratings of faces presented in the pleasant odour conditions. This finding could have been due to increased allocation of attention to an unpleasant stimulus, as discussed above (T. A. Ito et al., 1998). It has been noted that unpleasant odours induce negative emotional reactions (e.g. disgust), whilst pleasant odours rarely induce intense emotional reactions (e.g. euphoria) (Mackay-Sim & Royet, 2006). This phenomenon may be responsible for the lack of pleasant odour effects. Previous studies have also found that ratings of neutral faces were affected by unpleasant odours, but not pleasant odours or clean air (Dematte et al., 2007), and that neutral faces were subject to aversive conditioning with unpleasant odours, but not to appetitive conditioning with pleasant odours (Herrmann et al., 2000). The lack of a pleasant odour priming effect may have been due to the fact that the pleasantness rating of the pleasant odour was not as high as the unpleasantness rating of the unpleasant odour, a finding that was also reported by Herrmann et al. (2000). As a result, the salience of the pleasant odour may have been lower than that of the pleasant odour and therefore less likely to influence face ratings. Interestingly, an unpleasant odour has been shown to boost loss aversion and increase the skin conductance response to losses in a monetary gamble task, whilst a pleasant odour failed to affect either measure (Stancak et al., 2015), suggesting a greater capacity of unpleasant than pleasant odours to alter hedonic evaluations.

In summary, the results suggest that unpleasant odours are able to influence hedonic evaluations of faces both with and without a temporal lag between the odour and the face, but that odour priming is stronger with simultaneous stimulation. Such an effect was apparent in subjective evaluations, cortical potentials and even in the respiratory pattern. Unpleasant odours signal a danger such as fire, poisons, or spoiled food. A stronger priming effect of unpleasant odours for concurrently occurring visual stimuli compared to stimuli occurring later may help to shape a more robust and focused behavioural response by tuning the hedonic evaluation of

the visual stimulus towards the unpleasant pole. Such multimodal effects may allow for prompt mobilisation of behavioural resources to tackle potential danger.

Chapter 5

Pleasant and unpleasant odour-face combinations influence face and odour perception: an event-related potential study.

This experiment investigated the effects of pleasant and unpleasant odours and happy and disgusted faces on facial expression perception and odour pleasantness and intensity perception, using EEG.

The manuscript is currently in preparation for submission to Biological Psychology.

The roles of the co-authors are summarised below:

I designed the study in collaboration with Andrej Stancak, and collected the data. Katerina Kokmotou, Vicente Soto, and Nicholas Fallon assisted with the data collection. Andrej Stancak and Nicholas Fallon provided training during the data analysis. I analysed the data, interpreted the results, and wrote the manuscript. Katerina Kokmotou, Vicente Soto, Nicholas Fallon, Anna Thomas, Timo Giesbrecht, Matt Field and Andrej Stancak provided useful comments on the manuscript.

5.1 Abstract

Neural mechanisms underlying the effects of congruent and incongruent odours on facial expression perception are not clear. Moreover, the influence of emotionally-valenced faces on odour perception is not established. To further explore such effects, we investigated the effects of pleasant and unpleasant odours paired with happy and disgusted faces on subjective ratings and event-related potential (ERP) responses to faces.

Participants rated the pleasantness of happy and disgusted faces that appeared during 3 second pleasant (jasmine) or unpleasant (methylmercaptan) odour pulses, or without odour. Odour pleasantness and intensity ratings were also recorded in each trial. EEG was recorded continuously using a 128-channel EGI (Electrical Geodesics, Inc., USA) system.

Results indicated reciprocal effects of valenced odours and emotional faces. Specifically, disgusted faces presented in the unpleasant odour condition were rated less pleasant than the same faces presented in the pleasant or no odour conditions. Both pleasant and unpleasant odours were rated as more pleasant when paired with happy faces, and the unpleasant odour was rated as more intense when paired with disgusted faces. Odour-face interactions were evident in the N200 and N400 ERP components: Odour-face congruency effects were apparent in the unpleasant odour condition, whilst pleasant odour masked such effects. Unpleasant odour paired with disgusted faces resulted in a decrease in inspiration.

Congruent pairings of unpleasant odour and disgusted faces resulted in stronger shifts in face evaluation, changes in ERP responses to faces, increased odour intensity ratings and a decrease in inspiration. These findings likely represent a heightened adaptive response to unpleasant stimuli presented across multiple modalities, prompting appropriate behaviour in the presence of danger. Pleasant odour masked congruency effects in ERPs, suggesting that the hedonic state induced by a pleasant odour may reduce any such response.

5.2 Introduction

Previous research has shown that odours modulate face processing and recognition (Steinberg et al., 2012; Walla, 2008), subjective ratings of faces (Bensafi, Pierson, et al., 2002; Cook et al., 2015; Dematte et al., 2007; Li et al., 2007; McGlone et al., 2013; Seubert et al., 2014), and perceptions of facial expressions (Leleu, Demily, et al., 2015; Leppanen & Hietanen, 2003; Pause et al., 2004; Seubert et al., 2010; Zhou & Chen, 2009). The effects of odours on perception of facial expressions are often driven by affective congruency between odours and faces. For example, Leppanen and Hietanen (2003) observed that happy faces were recognised faster than disgusted faces in the presence of a pleasant odour. Moreover, Leleu, Demily, et al. (2015) observed that the minimum amount of visual information required to perceive an expression was lowered when the odour context was emotionally congruent.

More recently, the effect of odours on perception of facial expressions has been investigated using EEG, but the influence of congruency on such effects is less clear. One study showed that both neutral and negatively-valenced chemosensory signals modulated N170 amplitudes in responses to fearful facial expressions (Adolph et al., 2013). Another observed that stress sweat odour enhanced the late LPP in responses to neutral and ambiguous faces (Rubin et al., 2012). Leleu, Godard, et al. (2015) found that an aversive olfactory context modulated the P200 by amplifying the difference in responses to neutral versus happy and disgusted facial expressions. In these previous experiments, there were no explicit tasks regarding the facial expressions or olfactory stimuli. Whether the effects of congruent and incongruent odour-face interactions on EEG activity are also related to subjective ratings of facial expressions has yet to be investigated. Doing so will contribute to our understanding of the neural mechanisms underlying olfactory-visual influences on behaviour.

In addition to the effects of odours on perceptions of visual stimuli, reciprocal effects (i.e. the effects of visual stimuli on odour perception) are also well documented (Dematte et al., 2009; Gottfried & Dolan, 2003; Olofsson et al., 2012; Pollatos et al., 2007; Seo, Arshamian, et al., 2010). Importantly, some studies have demonstrated that visual information can affect odour pleasantness and intensity perception. Neutral odours were rated less pleasant and more intense following

unpleasant picture presentation, and more pleasant after viewing positive images (Pollatos et al., 2007). Another study showed that congruent symbol-odour pairs increased perceived pleasantness and intensity of a pleasant odour, and increased the unpleasantness of an unpleasant odour. Furthermore, congruent odour-symbol pairs produced higher amplitudes in olfactory ERPs (Seo, Arshamian, et al., 2010). It is clear that visual information can affect odour perception and that congruency plays a role, however, the effects of congruent and incongruent facial expressions on evaluations of odour pleasantness and intensity have not yet been investigated. Both face and odour processing almost always involve some aspect of emotion (Walla, 2008). Investigating bidirectional cross-modal effects of odours and emotional faces will provide further understanding of olfactory-visual integration in the context of emotion.

The aim of the present study was to investigate the effects of pleasant and unpleasant odours paired with happy and disgusted faces on evaluations of the facial expressions and odour pleasantness and intensity. Our study was the first of its kind to observe effects of olfactory-visual interactions on perceptions of both the visual and odour stimuli, using ERP analysis. Given the previous findings (Leleu, Demily, et al., 2015; Leppanen & Hietanen, 2003; Seo, Arshamian, et al., 2010), we hypothesised that congruent odour-face pairings would shift face and odour pleasantness ratings further in the direction of the given odour-face valence, and increase intensity ratings of odours. Moreover, in line with previous results (Cook et al., 2015; Leleu, Godard, et al., 2015; Rubin et al., 2012), we expected odour-face interactions to affect the P200 and LPP components of the ERP during face processing. The present study also contributes to a more general literature on evaluative priming (Herring et al., 2013). Using odours and faces as both primes and targets, we aimed to extend the current understanding of the mechanisms underlying evaluative priming by examining the phenomenon in a cross-modal sense, using ERP analysis.

5.3 Methods and Materials

5.3.1 Participants

A total of 25 (11 male) healthy participants aged 18−30 years (mean ± standard deviation: 23.28 ± 3.58) took part in the experiment after giving written informed consent in accordance with the Declaration of Helsinki. The study was approved by the Research Ethics Committee at the University of Liverpool. Two participants exercised their right to withdraw from the experiment for personal reasons, and data from a further three participants were subsequently excluded from the EEG analysis due to excessive amounts of artifacts. Hence, behavioural data from 23 (10 male) participants, and EEG data from 20 (9 male) participants were used in the analysis. All participants were initially screened in a separate session using the Sniffin'Sticks (Hummel et al., 1997) test battery to ensure adequate odour identification ability. Participants were asked not to smoke, drink coffee or chew gum for two hours prior to the experiment, and were asked to minimise their use of fragranced products on the day. Participants were reimbursed for their time and travel expenses.

5.3.2 Visual and olfactory stimuli

Face-images of 30 actors (15 male) showing happy and disgusted expressions were used in the experiment, for a total of 60 faces. These were selected from the NimStim Set of Facial Expressions (Tottenham et al., 2009). All face images were frontal views, in colour, with a consistent light background and similar dimensions.

Odours were administered through two tubes approximately two centimetres away from the nostrils; using a custom-built, continuous airflow, computer-controlled olfactometer with 8 channels (Dancer Design Ltd., UK). Odour pulses were embedded within a constant flow of clean air, in order to avoid effects of a sudden increase in airflow associated with presentation of an odour (Huart et al., 2012). Airflow was kept constant at 2.5 l/min.

There were three odour conditions in the experiment; pleasant, unpleasant and a neutral, 'clean air' control. Methylmercaptan (1% dilution in Propylene Glycol), a rotten cabbage-like odour, was selected for the unpleasant condition. Jasmine odour (no dilution) was selected for the pleasant condition. These dilutions were matched on perceived intensity based on data from previous experiments (Cook et al., 2015; Cook et al., under review). Odours were supplied by Symrise Ltd. (Netherlands). Propylene Glycol (1,2-Propanediol 99%, Sigma-Aldrich Ltd., UK) was used for dilution, the clean air control and constant flow.

Both presentation of the experimental task stimuli and triggering of the odour valves were achieved using the Cogent 2000 v. 1.32 program (Wellcome Department of Imaging Neuroscience, United Kingdom) running in Matlab v. R2011a (The MathWorks, Inc., USA). In between experimental blocks and sessions, a Blueair 203 air purifier (Blueair Ltd., Sweden) was used to minimise any residual odour that may have carried into the next experimental block or session.

5.3.3 Recordings

EEG was recorded continuously using a 128-channel Geodesics EGI System (Electrical Geodesics, Inc., Eugene, Oregon, USA) with a sponge-based Geodesic Sensor Net. The sensor net was aligned with respect to three anatomical landmarks; two pre-auricular points and the nasion. Electrode-to-skin impedances were kept below 50 kΩ and at equal levels across all electrodes. The recording band-pass filter was 0.01−1000 Hz, and the sampling rate was 1000 Hz. Electrode Cz was used as the reference.

Participants' respiration and pulse rate were recorded continuously throughout the experiment with a piezoelectric respiratory belt transducer worn around the chest at the level of the epigastrium, and a finger pulse oximeter transducer worn on the index finger of the left hand (ADInstruments Ltd., Oxford, UK). Signals were transduced and extracted using LabChart 7 (ADInstruments Ltd., Oxford, UK).

5.3.4 Procedure

After application of the EEG cap, participants were seated in a dimly lit, sound attenuated room facing a 19 inch LCD monitor (60 Hz refresh rate) placed approximately 0.7 m in front of them. First, the respiratory and pulse monitoring equipment was fitted onto participants and the signals were checked. Following this, the olfactometer head piece was fitted, and participants were given instructions. The experimental session lasted around 1.5 hours in total, including baseline odour ratings and the experimental task. Ratings of odour pleasantness, intensity, and familiarity were recorded before and after the task. Each odour was administered individually, in a four-second pulse manually triggered to coincide with the onset of inspiration. After each odour pulse, on-screen visual analogue scales prompted participants to rate the pleasantness (from 0 – very unpleasant to 100 – very pleasant), intensity (0 – no odour to 100 – very intense odour) and familiarity (0 – not familiar at all to 100 – extremely familiar) of the odour.

The experimental task was split into four blocks of 45 trials (180 trials in total). Trials were pseudo-randomly ordered such that each of the 30 actors appeared 6 times: showing a happy and a disgusted expression under each of the three odour conditions. A given actor never appeared showing the same expression more than once in each block. Odour presentation was also pseudo-random, such that all three odours were presented across all four blocks, but no two consecutive trials used the same odour. Figure 5.1 shows a flowchart of the trial procedure. Each trial began with a resting interval during which participants viewed a white cross on a black background. The duration of this interval was dependent upon the triggering of the odour pulse; the experimenter observed participants' respiratory waveforms, and manually triggered the odour pulses at the very onset of inspiration. Odour pulses were 3000 ms in duration. At a random time point between 1000–2000 ms of the odour pulse, a happy (half of the trials) or disgusted face was displayed on-screen for 300 ms. Following the odour pulse, a 3000 ms resting interval with a black screen preceded a rating scale prompting participants to rate the pleasantness of the facial expression (from 0 – very unpleasant to 100 – very pleasant). Once they had responded, a second screen with two scales prompted participants to rate the pleasantness (from 0 – very unpleasant to 100 – very pleasant) and the intensity (0 –

no odour to 100 – very intense odour) of the odour administered in that trial. After their response, the next trial began.

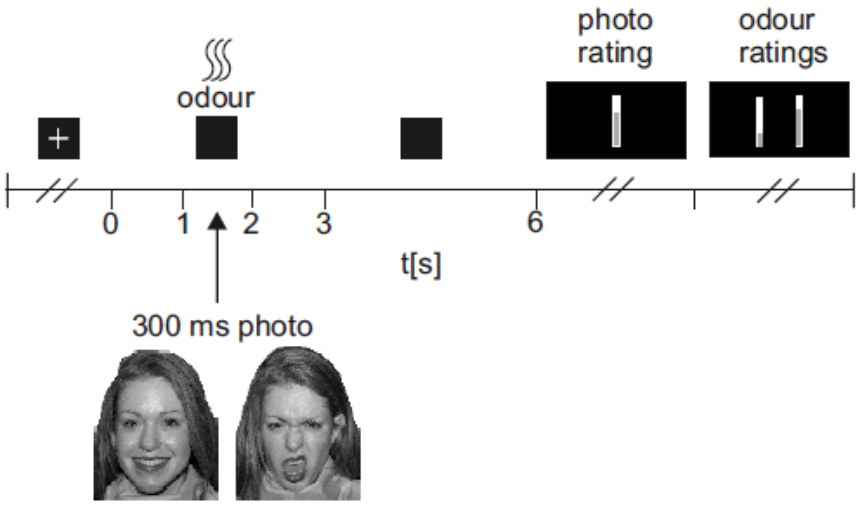

Figure 5.1: Flowchart of experimental trial procedure.

5.3.5 Behavioural analysis

Ratings of odour pleasantness, intensity and familiarity taken before and after the experimental task were collapsed and analysed using paired t-tests. Data from the experimental task were analysed using 2 × 3 repeated measures ANOVAs, observing differences in face pleasantness ratings, and odour pleasantness and intensity ratings with odour condition (pleasant, unpleasant, neutral) and face type (happy or disgusted) as the independent variables. Significant main effects were investigated using pairwise comparisons; significant interactions were followed up with post-hoc t-tests and one-way ANOVAs, using Bonferroni correction for multiple comparisons. P values in all ANOVA effects were adjusted using the Greenhouse-Geisser ε method. All behavioural data was analysed using SPSS v. 22 software package (IBM Inc., USA).

5.3.6 ERP analysis

EEG recordings were pre-processed using BESA v. 6.0 (MEGIS GmbH, Germany). Data were first referenced to a common average using the common averaging method (Lehmann, 1987). The oculographic and, when necessary, electrocardiographic artifacts were removed by principal component analysis (Berg & Scherg, 1994). Data were visually inspected for the presence of any movement or muscle artifacts, and trials contaminated with artifacts were excluded. The mean number of accepted trials across all subjects and all conditions was 161 (± 17.02). Participants were excluded from the analysis if the number of trials accepted was less than 127 (2 standard deviations from the mean).

Data were band-pass filtered from 2−35 Hz and down-sampled to a rate of 256 Hz, and exported from BESA into the SPM12 software package (Statistical Parametric Mapping, UCL, England; http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Event-related potentials (ERPs) in response to faces were computed separately for each odour and face condition by averaging respective epochs in the intervals ranging from 300 ms before photo onset to 1000 ms after photo onset. The baseline period ranged from -300 ms to 0 ms relative to the onset of the visual stimulus.

We applied an omnibus analysis of the effects of odours on ERPs involving all time points from 0 ms to 1000 ms and all scalp sites, allowing us to explore the effects of odours on ERPs without applying a priori knowledge of peak latencies. The SPM12 toolbox combines advanced statistical models with robust control for Type I error (Poline et al., 1997; Kiebel & Friston, 2004). In contrast to alternative approaches, such as permutation analysis of clusters of ERPs over the epoch time (Maris & Oostenveld, 2007), SPM applies the theory of random fields to volumes of space-time data. This allows for calculation of the degrees of freedom in the evaluation of statistical test results based on the spatial and temporal complexity of data (Worsley, 2003).

The statistical analysis was performed in two steps. In the initial exploratory step, EEG data were converted into three-dimensional scalp-time images using SPM. The electrodes were mapped onto a standardised scalp grid sized 32 × 32 pixels

(pixel size 4.25 × 5.3 mm^2), representing the field potential planes stacked over the time axis. Images were smoothed with a Gaussian kernel of 9 × 9 × 20 mm^2 .ms (full width at half maximum). Data from over the whole epoch (385 time samples) and all standardised scalp points were screened for statistically significant effects of odours and face-valence using a flexible factorial ANOVA for repeated measures. The flexible factorial model in SPM allows for the inclusion of the subject factor as an independent variable. We applied an uncorrected threshold of $P < 0.001$, and a cluster size threshold of 20 contiguous space-time voxels to detect clusters affected by odours and face-valence. The data was masked such that only clusters occurring later than 100 ms following face onset were analysed. The amplitude data from these clusters were subsequently analysed using further repeated measures ANOVAs in SPSS v. 22 (IBM Inc., USA). The statistical threshold of this confirmatory analysis was $P < 0.05$.

5.3.7 Analysis of respiratory movements

Respiratory movement signals were low-pass filtered, and averaged separately for each of the six conditions in the epoch of interest, then analysed statistically using a 2 × 3 repeated measures ANOVA (2 face types, 3 odours). The 7 s analysis epoch ranged from odour onset (t = 0 s) to 7 s after odour onset. Therefore, the interval 1–3 s coincided with the ERP analysis epoch. To control for Type I error due to the large number of ANOVAs, given that one ANOVA was computed on each time sample, a permutation analysis with 500 permutations was used to correct the P values (Maris & Oostenveld, 2007). Data from the interval showing a significant effect of condition on respiratory movements were analysed using confirmatory repeated measures ANOVA in SPSS. We used a 2 × 3 ANCOVA for repeated measures in BMDP 2V program (Biomedical Data Package, Cork, Ireland) to analyse whether changes in respiratory movement patterns contributed to the effects of experimental condition observed in ERP clusters.

5.4 Results

5.4.1 Odour ratings

Mean ratings of odour pleasantness, intensity and familiarity taken before and after the experimental task were collated and are shown in Table 5.1. A paired t-test confirmed that jasmine was rated as significantly more pleasant than methylmercaptan ($t(22) = 21.55$, $P < 0.001$). A further paired t-test showed there was no significant difference between intensity ratings of jasmine and methylmercaptan ($t(22) = -1.58$, $P = 0.13$). A third t-test confirmed that there was no significant difference in familiarity ratings of jasmine and methylmercaptan ($t(22) = 1.14$, $P = 0.27$).

Table 5.1: Mean (± standard deviation) ratings of odour pleasantness, intensity and familiarity that were taken before and after the experimental task and concatenated.

	Pleasantness	Intensity	Familiarity
Jasmine	79.28 (± 6.97)	71.95 (± 7.67)	71.67 (± 15.48)
Methylmercaptan	15.7 (± 11.94)	76.34 (± 13.08)	66.59 (± 19.23)

5.4.2 Face and odour ratings

Figure 5.2A shows the mean ratings of the happy and disgusted faces under each odour and face condition. A repeated-measures ANOVA revealed a significant main effect of odour on ratings of faces overall ($F(2, 44) = 30.4$, $\eta_p^2 = 0.58$, $P < 0.001$). Pairwise comparisons indicated that all faces presented in the methylmercaptan odour condition were rated as less pleasant (44.68 ± 26.49) in comparison to faces presented in both the clean air (48 ± 25.47) and jasmine (49.2 ± 26.43) conditions ($P < 0.001$), and faces in the jasmine condition were rated as significantly more pleasant than those in the clean air condition ($P = 0.01$). There was a significant main effect of face type on ratings of faces ($F(1, 22) = 886.37$, $\eta_p^2 =$

0.98, P < 0.001), confirming that happy faces were rated as significantly more pleasant (72.55 ± 1.1) than disgusted faces (22.04 ± 1.09). There was also a significant interaction between odours and face type affecting face ratings (F(2, 44) = 4.28 η_p^2 = 0.16, P = 0.02). Post-hoc one-way ANOVAs were employed to investigate this interaction, by observing the effects of odours on face ratings of happy and disgusted faces separately. For happy faces, a one-way ANOVA revealed a significant effect of odour (F(2, 44) = 18.83, η_p^2 = 0.46, P < 0.001). Pairwise comparisons indicated that happy faces presented in the jasmine odour condition were rated as more pleasant (74.78 ± 5.77) in comparison to the same faces presented in both the clean air (72.64 ± 5.31, P = 0.002) and methylmercaptan (70.24 ± 5.89) odour conditions (P < 0.001), and happy faces in the methylmercaptan condition were rated as significantly less pleasant than those in the clean air condition (P = 0.001). For disgusted faces, a one-way ANOVA revealed a significant effect of odour (F(2, 44) = 28.29, η_p^2 = 0.56, P < 0.001). Pairwise comparisons indicated that disgusted faces in the methylmercaptan condition were rated significantly less pleasant (19.12 ± 5.85) than the same faces in both the clean air (23.38 ± 5.52) and jasmine (23.62 ± 5.26) odour conditions (P < 0.001). There was no significant difference in ratings of disgusted faces between the jasmine and clean air conditions (P > 0.05).

Figure 5.2B shows the mean odour pleasantness ratings from experimental trials for each odour and face condition. A repeated-measures ANOVA revealed a significant main effect of odour type on odour pleasantness ratings (F(2, 44) = 323.76, η_p^2 = 0.94, P < 0.001). Pairwise comparisons confirmed that the jasmine odour was rated as more pleasant (74.56 ± 7.75) than both clean air (51.87 ± 3.19) and methylmercaptan (20.55 ± 7.45, P < 0.001); and that methylmercaptan was also rated as significantly less pleasant than clean air (P < 0.001). There was also a significant main effect of face type on odour pleasantness ratings (F(1, 22) = 12.29, η_p^2 = 0.36, P = 0.003), indicating that all odours were rated as more pleasant (49.67 ± 23.15) when presented with happy faces in comparison to when presented with disgusted faces (48.31 ± 23.27). The interaction between odours and face type

affecting odour pleasantness ratings did not reach statistical significance ($F(2, 44) = 2.34$ $\eta_p^2 = 0.1$, $P = 0.11$).

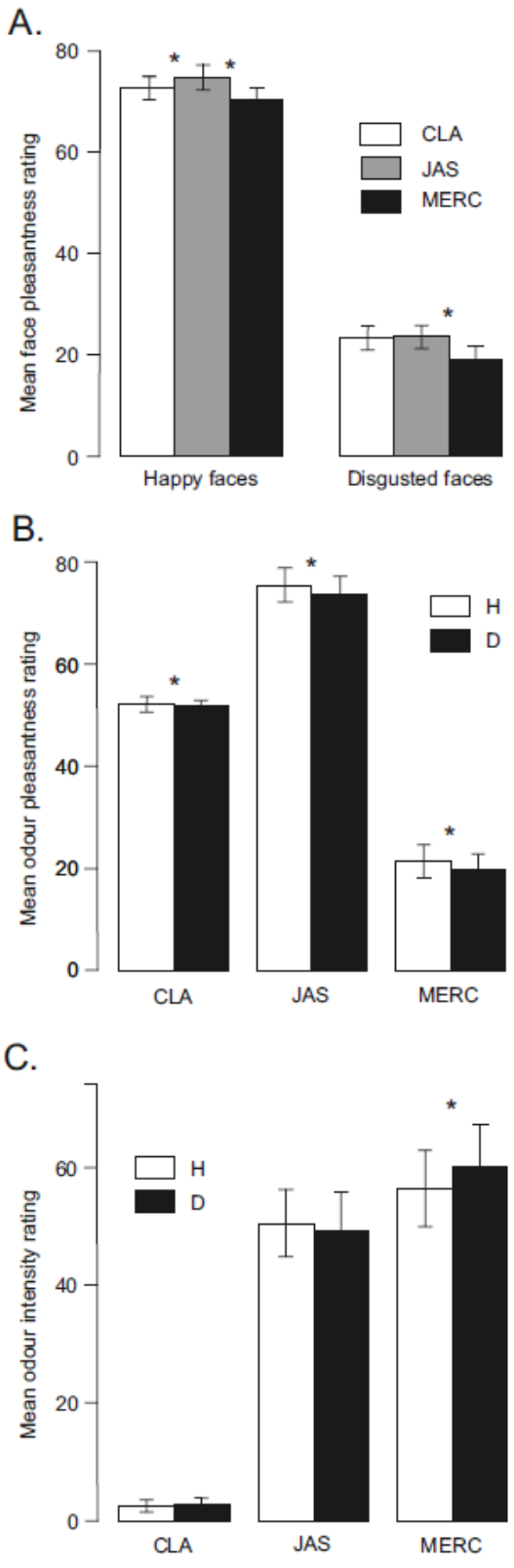

Figure 5.2: Mean ratings of face pleasantness, odour pleasantness and odour intensity. (A) Bar graph illustrating the mean ratings of face pleasantness in each odour and face condition. White bars represent clean air trials (labelled CLA), grey bars represent trials using jasmine odour (labelled JAS), and black bars represent trials using methylmercaptan (labelled MERC). Asterisks indicate statistically significant differences between odour conditions ($P < 0.025$). (B) Bar graph illustrating mean ratings of odour pleasantness in each odour and face condition. White bars represent trials where happy faces were presented (labelled H), and black bars represent trials where disgusted faces were presented (labelled D). Odour conditions are labelled CLA, JAS, MERC. Asterisks indicate significant differences between happy and disgusted face conditions ($P < 0.025$). (C) Bar graph illustrating mean ratings of odour intensity in each odour and face condition. White bars represent trials where happy faces were presented (labelled H), and black bars represent trials where disgusted faces were presented (labelled D). Odour conditions are labelled CLA, JAS, MERC. Asterisks indicate statistically significant differences between happy and disgusted face conditions ($P < 0.025$).

Figure 5.2C shows the mean odour intensity ratings from experimental trials for each odour and face condition. A repeated-measures ANOVA revealed a significant main effect of odour type on intensity ratings ($F(2, 44) = 219.26$ $\eta_p^2 = 0.91$, $P < 0.001$). Pairwise comparisons confirmed that methylmercaptan was rated as more intense (58.25 ± 15.45) than both jasmine (49.94 ± 13.67, $P = 0.003$) and clean air (2.63 ± 2.49, $P < 0.001$); and that jasmine was also rated as significantly more intense than clean air ($P < 0.001$). There was no significant main effect of face type ($P > 0.05$); however, there was a significant interaction between odour and face type affecting odour intensity ratings during experimental trials ($F(2, 44) = 6.89$, $\eta_p^2 = 0.24$, $P = 0.003$). Post-hoc t-tests confirmed that this effect was driven by intensity ratings of methylmercaptan: when presented in combination with disgusted faces, methylmercaptan was rated as significantly more intense (60.11 ± 16.38) than the same odour presented with happy faces (56.39 ± 14.96, $t(22) = -3.34$, $P = 0.003$). There were no significant effects of face type on intensity ratings of clean air or jasmine ($P > 0.05$).

5.4.3 ERP components

Figure 5.3 illustrates the event-related potentials in response to faces across all trials and all conditions in the form of a butterfly plot and topographic maps of selected potential components. The topography of the first component showed bilateral positivity over the occipital electrodes and negativity over frontal electrodes, peaking around 95 ms. This is consistent with characteristics of the P1 component, which is related to early processing of visual stimuli (Hopf et al., 2002). The second component, peaking around 145 ms, showed negative potential over parietal and temporal electrodes, consistent with characteristics of the N170 face-processing component (Bentin et al., 1996). The next component peaked around 200 ms, showing positive potential in parietal-occipital, and strong negative potential in central-frontal electrodes, consistent with typical characteristics of the N200 component (Folstein & Van Petten, 2008). The fourth component peaked at 395 ms and showed weak positivity in occipital electrodes.

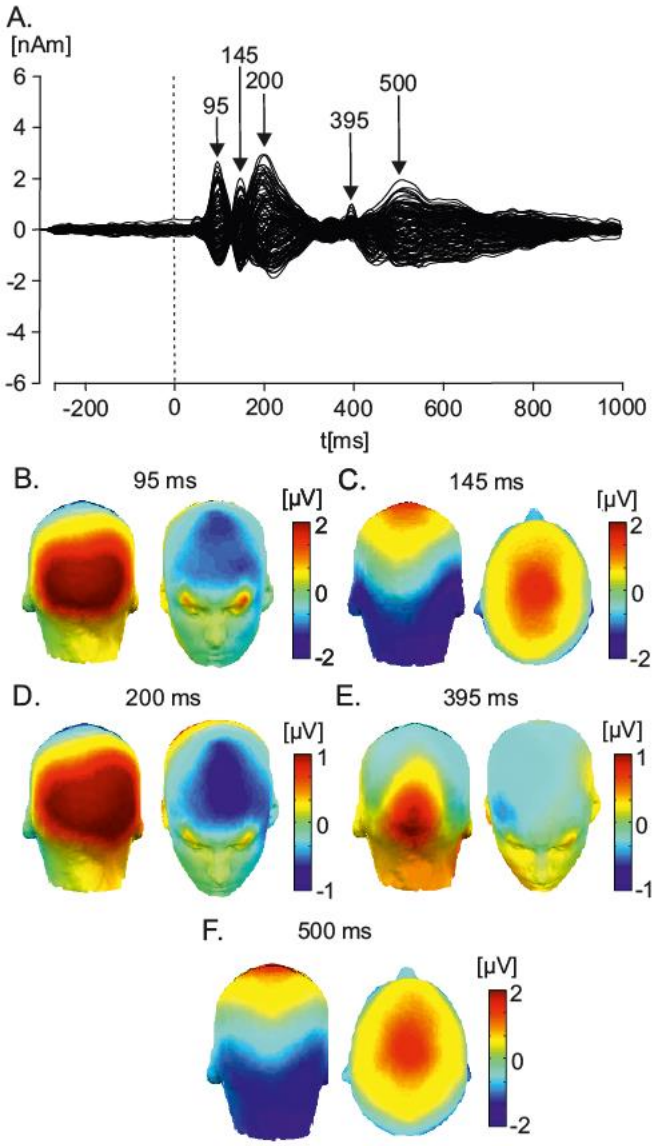

Figure 5.3: Butterfly plot of grand average ERP response to faces and corresponding scalp topographies. (A) Butterfly plot of grand average ERPs in response to faces. Peak latencies of distinct ERP components (95 ms, 145 ms, 200 ms, 395 ms and 530 ms) are highlighted with arrows. (B) Latency component 95 ms (P1). The topographic maps of grand average ERPs overlaid on the volume rendering of the human head are shown. (C) Latency component 145 ms (N170). (D) Latency component 200 ms. (E) Latency component 395 ms (N400). (F) Latency component 500 ms (LPP).

The final component was a long-latency component peaking around 500 ms, showing a strong negative potential over occipital and parietal electrodes, and a positive potential over central midline electrodes. These components are consistent with characteristics of the N400 component, implicated in the processing of meaningful stimuli, including faces (Kutas & Federmeier, 2011), and the late positive potential (LPP), which is sensitive to the emotional content of pictures,

words and faces (Cacioppo et al., 1993; Cuthbert et al., 2000; Hajcak et al., 2007; Hajcak et al., 2006).

5.4.4 Effects of odours and face-valence on ERPs

SPM12 was used to compute a 2 × 3 (face valence × odour) repeated measures ANOVA on smoothed scalp-time images of data from 0−1000 ms relative to the onset of the faces. The ANOVA revealed scalp-time clusters showing significant main and interaction effects of face-valence and odour on the ERP response to faces. Figure 5.4 illustrates these significant scalp-time clusters. The corresponding topographic maps from each odour/face condition for each significant cluster are shown with bar graphs representing the mean EEG scalp-amplitude (μV).

There was a significant main effect of face valence on ERP responses to faces at 192 ms and 704 ms after face onset (uncorrected $P < 0.001$), coinciding with the N170 component and the late-LPP, respectively (see Figure 5.4A). Subsequent t-tests performed on EEG amplitude data from these two clusters showed that happy faces yielded stronger EEG amplitude than disgusted faces in both the 192 ms cluster ($t(19) = -5.01$, $P < 0.001$), and the 704 ms cluster ($t(19) = -2.91$, $P = 0.009$).

Another statistically significant scalp-time cluster represented a main effect of odour on ERP responses to faces at 165 ms following face onset (unc. $P < 0.001$), in frontal electrodes during the N170 component (see Figure 5.4B). A confirmatory one-way ANOVA in this cluster showed a significant effect of odour ($F(2, 38) = 16.84$, $\eta_p^2 = 0.47$, $P < 0.001$). Pairwise comparisons indicated that there were significant differences in EEG amplitude between all three odour conditions ($P < 0.05$): irrespective of face-valence, faces in the clean air condition produced a small negative amplitude (-0.24 ± 0.76), faces in the pleasant odour condition produced a very small negative amplitude (-0.1 ± 0.68), and faces in the unpleasant odour condition produced a positive amplitude (0.27 ± 0.95).

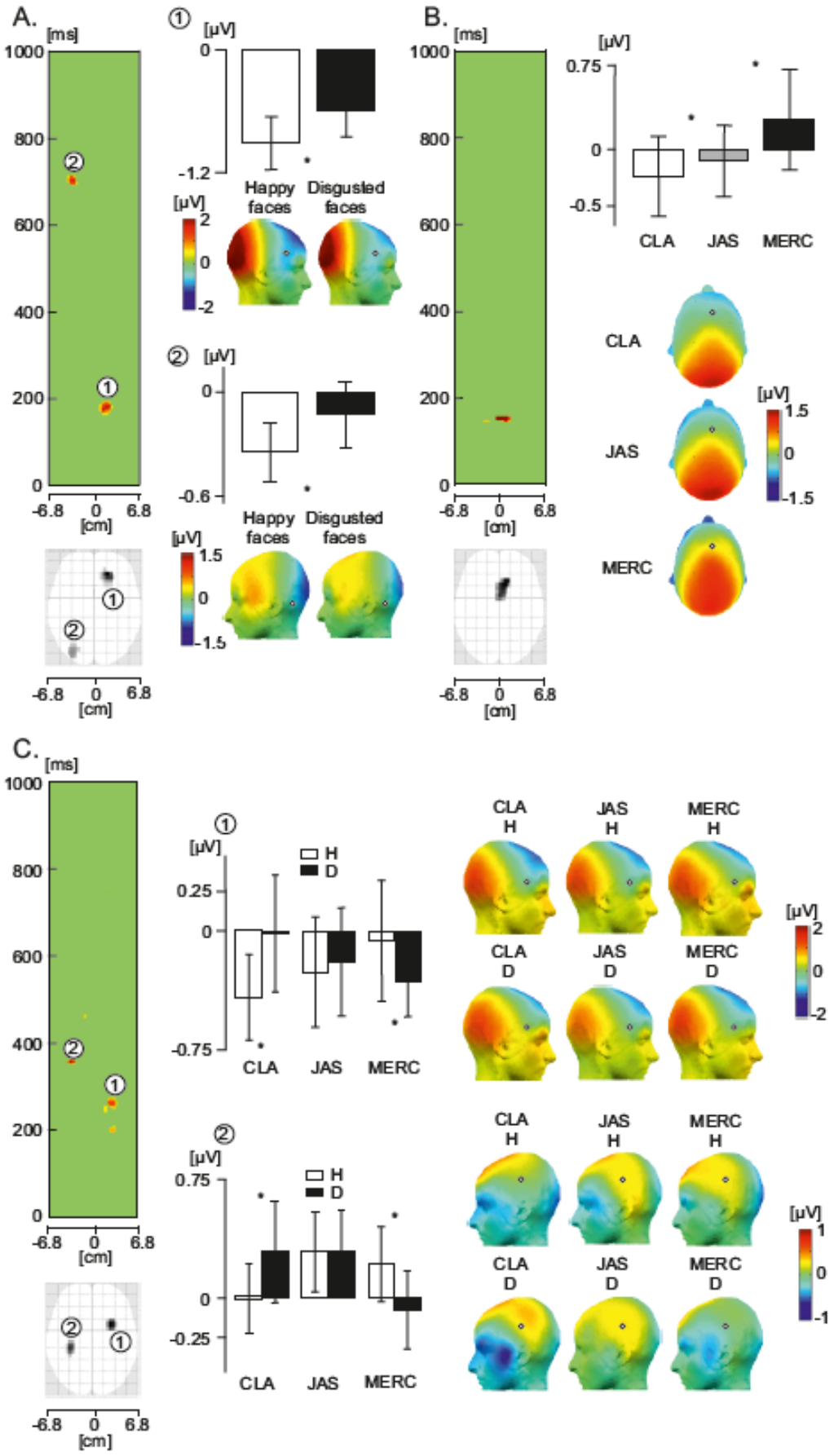
A.
[ms]
1000
800
600
400
200
0
-6.8
0
6.8
[cm]
①
②
[μV]
0
-1.2
Happy faces
Disgusted faces
2
-2
-0.6
1.5
-1.5
B.
0.75
-0.5
CLA
JAS
MERC
C.
H
D
0.25
-0.75
0.75
-0.25
CLA H
JAS H
MERC H
CLA D
JAS D
MERC D
1
-1

Figure 5.4: Repeated-measures ANOVA showing the effects of the three odour conditions and two face conditions on ERP responses to faces. (A) Main effect of face-valence on ERP responses to faces across all odour conditions. The green panel shows statistically significant latency periods (uncorrected P < 0.001) in the scalp-time plot where F values represent the strength of variance between SOA conditions over the horizontal axis of the scalp in every time sample from 0 ms and 1000 ms relative to the onset of the face photograph. The scalp values over the horizontal axis of the scalp are averages of F values occurring at each vertical point for a given horizontal point in the standardised scalp map (from -6.8 cm to +6.8 cm). There were two spatio-temporal clusters showing a statistically significant effect of face-valence. Below the green panel is the standard scalp map of statistically significant clusters using ERPs. The first significant cluster, labelled 1, occurred at 192 ms and had negative amplitude. The second, labelled 2, occurred at 704 ms and also had negative amplitude. Bar graphs illustrate the mean EEG amplitude for each cluster under each face condition (μV). White bars represent trials with happy faces, and black bars represent trials with disgusted faces. Asterisks indicate statistically significant differences between happy and disgusted face conditions (P < 0.05). Corresponding topographic maps of the numbered significant clusters for the two SOA conditions are shown. White circles with a black outline pinpoint the location of the significant electrode clusters. (B) Main effect of odour condition on ERP responses to faces across both face conditions. The green panel shows statistically significant latency periods (P < 0.05 FWE) in the scalp-time plot where F values represent the strength of variance between odour conditions. One spatio-temporal cluster showed a statistically significant effect of odour around the N170 component. Below the green panel is the standard scalp map of the statistically significant cluster. Bar graphs illustrate the mean EEG amplitude at this cluster under each odour condition (μV). The white bar represents the clean air condition (labelled CLA), the grey bar represents the pleasant odour condition (labelled JAS) and the black bar represents the unpleasant odour condition (labelled MERC). Asterisks indicate statistically significant differences between odour conditions (P < 0.05). Corresponding topographic maps of the significant cluster for the three odour conditions are shown. (C) Interaction between odour and face-valence condition affecting ERP responses to faces. The green panel shows statistically significant latency periods (P < 0.001 uncorrected) in the scalp-time plot. Two spatio-temporal clusters during the LPP showed were significantly affected by an interaction between odour and face-valence conditions. Below the green panel is the standard scalp map of the statistically significant clusters. The first significant cluster, labelled 1, occurred at 259 ms and had negative amplitude. The second, labelled 2, occurred at 352 ms and had positive amplitude. Bar graphs illustrate the mean EEG amplitude for each cluster under each condition (μV). White bars represent trials with happy faces, and black bars represent trials with disgusted faces. Odour conditions are labelled CLA, JAS, and MERC. Asterisks indicate statistically significant differences between happy and disgusted face conditions (P < 0.025). Corresponding topographic maps of the numbered significant clusters for all conditions are shown.

An interaction between odour and face-valence yielded a significant effect on ERP responses to faces in two scalp-time clusters (unc. P < 0.001, see Figure 5.4C). One such interaction occurred at 259 ms following face onset ($F(2, 38) = 7.77$, $\eta_p^2 = 0.29$, $P = 0.003$). Post-hoc t-tests were employed to further investigate this interaction. These showed that happy faces produced a significantly greater negative potential at right frontal electrodes (-0.42 ± 0.58) than disgusted faces (-0.02 ± 0.79) in the clean air condition ($t(19) = -3.63$, $P = 0.002$), and that disgusted faces produced a significantly greater negative potential (-0.32 ± 0.48) than happy faces (-0.06 ± 0.81) in the unpleasant odour condition ($t(19) = 2.19$, $P = 0.04$). There was no significant difference in the amplitude produced by happy and disgusted faces in the pleasant odour condition (P > 0.05). An interaction between odour and face valence

also occurred at 352 ms following face onset (F(2, 38) = 5.98, η_p^2 = 0.24, P = 0.01). Post-hoc t-tests showed that disgusted faces produced a greater positive potential at left frontal-parietal electrodes (0.29 ± 0.68) than happy faces (-0.01 ± 0.48) in the clean air condition (t(19) = -2.78, P = 0.01), and that happy faces produced a greater positive potential (0.21 ± 0.5) than disgusted faces (-0.76 ± 0.52) in the unpleasant odour condition (t(19) = 2.76, P = 0.01). There was no significant difference in ERP amplitudes produced by happy and disgusted faces in the pleasant odour condition (P > 0.05).

5.4.5 Respiratory movements

Figure 5.5A shows averaged respiratory waveforms for each condition in a 7 s interval, beginning at odour onset. A repeated-measures ANOVA (2 face-types, 3 odours) showed a statistically significant effect of odour during the interval 1530–2215 ms (P < 0.05), and a significant interaction between face valence and odour during the interval 1434–1796 ms (P < 0.05). Given that these intervals overlapped, it is likely that the main effect in the interval 1530–2215 ms was driven by the interaction during the interval 1434–1796 ms. To analyse these effects further, respiratory movement data from these intervals were subjected to repeated measures ANOVAs in SPSS. This confirmed a significant effect of odour on respiratory movements during the interval 1530–2215 ms (F(2, 38) = 3.53 η_p^2 = 0.16, P = 0.05), where pairwise comparisons confirmed a significant difference in respiratory movements between the jasmine and methylmercaptan odour conditions (P = 0.04). Inspiration was reduced during stimulation with methylmercaptan, compared to jasmine odour (see Figure 5.5A & 5.5B). Further analysis confirmed the interaction between odour and face valence during the interval 1434–1796 ms (F(2, 38) = 3.44, η_p^2 = 0.15, P = 0.05), and post hoc t-tests revealed that this interaction was representative of a marginally significant difference between respiratory movements in trials presenting happy faces compared to those presenting disgusted faces in the unpleasant odour condition only (t(19) = 1.8, P = 0.09). Inspiration was reduced

during presentation of disgusted faces compared to presentation of happy faces in the unpleasant odour condition (see Figure 5.5A & 5.5C).

Intervals showing significant effects of odour and face valence on respiratory movements overlapped with the period in which ERPs were recorded and analysed. However, repeated measures ANCOVA showed that there were no statistically significant covariate effects of respiratory movements on ERP data from any of the five significant scalp-time clusters ($P > 0.05$). Therefore, it is unlikely that differences in respiratory movements directly affected odour- or face-related ERP changes.

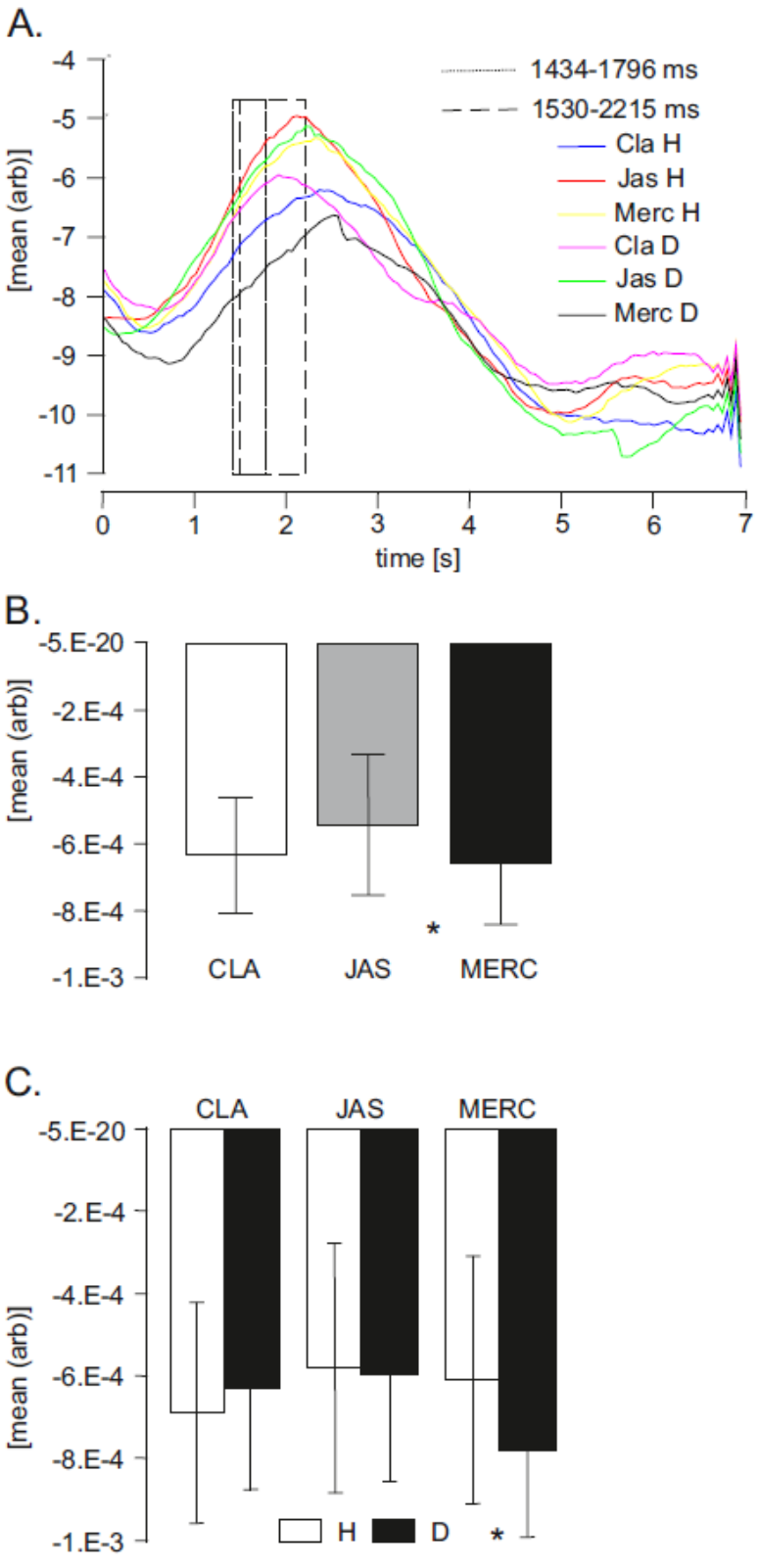

Figure 5.5: (A) Average respiratory waveforms for each condition. Respiratory movement signals from every subject across all trials were averaged over a period of 7 seconds, beginning at odour onset (Time 0). The blue line represents clean air trials using happy faces (denoted as 'Cla H'), the red line represents pleasant odour trials using happy faces ('Jas H') and the yellow line represents unpleasant odour trials using happy faces ('Merc H'). The pink line represents clean air trials using disgusted faces ('Cla D'), the green line represents pleasant odour trials using disgusted faces ('Jas D') and the black line represents unpleasant odour trials using disgusted faces ('Merc D'). Upwards deflection of respiratory signals corresponds to inspiration. The dashed line indicates the significant main effect of odour at 1530-2215 ms. The more solid line represents the significant interaction between odours and faces at 1434-1796 ms. (B) Mean respiratory amplitudes showing the main effect of odour condition during the interval 1530-2215 ms. An asterisk indicates the significant difference between the pleasant and unpleasant odour conditions ($P < 0.05$). (C) Mean respiratory amplitudes showing the interaction between odour and face conditions during the interval 1434-1796 ms. An asterisk indicates the marginally significant difference between happy and disgusted face conditions in the unpleasant odour condition ($P = 0.09$).

5.5 Discussion

Pleasant and unpleasant odours influenced evaluations of happy and disgusted facial expressions, and these effects were modulated by odour-face congruency. The effects were reciprocal: happy and disgusted faces also affected evaluations of odour pleasantness and intensity. Effects of odour-face interactions manifested in changes in cortical potentials during the N200 and N400 components of face ERPs. Moreover, respiratory movements were reduced when disgusted faces were presented in an unpleasant odour context.

Happy faces in the congruent, pleasant odour condition were rated as most pleasant, happy faces in the unpleasant odour condition were rated as least pleasant, and happy faces in the clean air condition were rated between the two. This finding corresponds with previous results showing that odour valence linearly modulated evaluations of neutral faces (Cook et al., 2015; Seubert et al., 2014). Disgusted faces were rated as significantly less pleasant when they were presented with a congruent, unpleasant odour, compared to the same faces paired with a pleasant odour or no odour. The lack of difference between ratings of disgusted faces in the clean air and pleasant odour conditions may be attributable to the stronger influence of a negative odour on evaluations. This is consistent with the negative bias hypothesis, which states that the influence of negative stimuli is often greater than the influence of positive stimuli of the same intensity (T. A. Ito et al., 1998; Smith et al., 2003; Smith et al., 2006). Indeed, previous studies have shown that unpleasant odours increase aversion to other unpleasant events, whereas pleasant odours had no effect (Stancak et al., 2015). The increase and decrease in pleasantness ratings of happy and disgusted faces paired with pleasant and unpleasant odours, respectively, further suggests that the congruency of odour-face valence has a role in the subjective evaluation of facial expressions (Leleu, Godard, et al., 2015; Leppanen & Hietanen, 2003). In particular, stronger subjective reactions to disgusted faces in the presence of an unpleasant odour may be characteristic of an evolutionarily adaptive response to combined aversive stimuli from visual and olfactory modalities.

Interestingly, regardless of valence, all odours were rated as more pleasant when paired with happy faces compared to when they were paired with disgusted faces. The unpleasant odour was also rated as more intense when paired with

congruent, disgusted face stimuli. These findings are consistent with previous results from studies using images and symbols as visual stimuli (Pollatos et al., 2007; Seo, Arshamian, et al., 2010), and novel in the respect that emotional faces were also able to induce such effects. Our results demonstrate not only that pleasant and unpleasant odour can influence continuous subjective evaluations of happy and disgusted faces, but also that emotional faces can affect perceptions of odour pleasantness and intensity. Congruency between odour-face pairs clearly has a role in these effects.

Odour-face interactions were observed during the N200 component of face ERPs. The N200 has been implicated in the analysis, discrimination and classification of visual stimuli (Naatanen & Picton, 1986; Ritter, Simson, & Vaughan, 1983). In the clean air condition, happy faces produced greater negative potential amplitude than disgusted faces. In the unpleasant odour condition, disgusted faces produced greater negative potential amplitude than happy faces, suggesting some congruency effects. In the pleasant odour condition, face valence did not differentiate the potential amplitude. A similar, but reversed effect was found in the N400 component, which is known to be involved in processing contextual information about faces (Kutas & Federmeier, 2011): Disgusted faces produced greater positive potential amplitude than happy faces in the clean air condition, and happy faces produced a greater positive potential amplitude than disgusted faces in the unpleasant odour condition, suggesting an effect of incongruity. Again, there was no difference in the amplitude produced by happy and disgusted faces in the pleasant odour condition.

Pleasant odour appeared to induce a moderate response to faces in both components, regardless of the face valence. A possible explanation for this is that the hedonic state induced by the pleasant odour was strong enough to mask any interactions with congruent or incongruent faces, whereas congruency effects took hold in the neutral and unpleasant odour conditions. Happy and disgusted faces may have been perceived as congruent or incongruent with clean air or unpleasant odour, and vice versa, resulting in increased cortical potentials for such congruent and incongruent pairings. These findings are consistent with those of Castle et al. (2000), who showed significant differences in the N400 for congruent versus incongruent stimuli in an unpleasant odour condition, but not in a pleasant odour condition. Our

results are also partially consistent with those of Leleu, Godard, et al. (2015), who found odour-face interactions during the P200, and showed that unpleasant odour context amplified the difference in responses to neutral versus happy and disgusted faces. However, their results suggested that unpleasant odour context increased responses to emotional faces in general, regardless of the face valence. On the other hand, our results suggest that unpleasant and no odour contexts amplified the difference between happy and disgusted faces, whilst pleasant odour context eliminated congruency effects in N200 and N400 components.

Recent studies from the more general evaluative priming literature suggest that evaluative incongruity is represented in the LPP and N400 components (Herring et al., 2011; Zhang, Li, Gold, & Jiang, 2010). Herring et al. (2011) argued that the N400 may be more specifically involved in semantic, rather than evaluative incongruity, and cross-modality priming. Our results showed an effect of incongruity during the N400, and therefore support and extend the finding that the N400 represents effects of congruency in cross-modal priming (Zhang et al., 2010). Encoding perspectives of evaluative priming suggest that primes activate object-evaluation associations in memory that make the valence of targets more accessible, thus facilitating evaluative priming. On the other hand, response perspectives suggest that primes influence the ease with which a person can generate a response to the target. A recent meta-analysis of evaluative priming studies argued that both encoding and response processes are involved in most cases, depending on the task (Herring et al., 2013). The present study observed effects of evaluative congruency in ERP responses to faces, likely an encoding phase, as well as in subjective behavioural responses. Our results therefore support the findings of Herring et al. (2013), and contribute that both encoding and response mechanisms were involved in evaluative priming where odours and faces served as cross-modal primes and targets.

An interesting odour-face interaction was also observed in the respiratory movement data. In the unpleasant odour condition, inspiration was significantly reduced during presentation of disgusted faces compared to happy faces. Decreased inspiration when an unpleasant olfactory stimulus was simultaneously paired with a congruent unpleasant visual stimulus is another example of the adaptive role of olfactory-visual integration in our multisensory environment. Indeed, aversive

odours act as a warning about dangers in our surroundings and evoke withdrawal reflexes (Stevenson, 2010). Evidently, this warning is heightened when an odour is accompanied by a congruent visual stimulus, resulting in decreased inspiration in the case of the present study. Previous studies showed enhanced skin conductance responses for unpleasant images combined with unpleasant odour (Banks et al., 2012), and decreased inspiratory time and breath duration for high arousal and unpleasant stimuli (Gomez et al., 2004; Ritz et al., 2000).

A main effect of odour, irrespective of face valence, was observed in the N170 component of face ERPs. The unpleasant odour produced the greatest positive potential amplitude, the pleasant odour produced very small negative amplitude, and clean air produced negative amplitude. The findings are partially consistent with those of Leleu, Godard, et al. (2015), who showed a generic enhancement of the EEG response to faces, regardless of their emotional content, between 130 and 180 ms after face onset when faces were presented with an odour. Moreover, results from our previous study showed an increase in N170 amplitude when faces were presented in the presence of an odour (Cook et al., under review). It is likely that faces presented in the unpleasant odour condition produced the largest N170 amplitude due to greater salience of the unpleasant odour. This is consistent with the aforementioned negative bias hypothesis (T. A. Ito et al., 1998), and may further represent an evolutionary adaptive response to aversive stimuli.

An effect of face valence, regardless of odour condition, was observed in the N170 and late-LPP components of face ERPs. Happy faces produced a stronger amplitude potential across all odour conditions than disgusted faces. Whilst previous studies have suggested that the N170 response is similar across faces, irrespective of emotional expression (Martin Eimer & Holmes, 2007; M. Eimer, Holmes, & McGlone, 2003), others have found differential effects depending on emotional expression (Batty & Taylor, 2003). The LPP is also known to be sensitive to the valence of pictures, words and faces (Cacioppo et al., 1993; Cuthbert et al., 2000; Hajcak et al., 2007; Hajcak et al., 2006). It is possible that happy faces resulted in increased cortical amplitude potentials due to a boosting effect of positive valence, in the same way that the pleasant odour context masked effects of congruency in odour-face interactions. We argue that happy faces may have had a greater activation effect

on reward circuitry or valuation structures in the brain (Lebreton et al., 2009). This may apply in particular to the cluster in the N170, as it was located in frontal electrodes and is thus more likely to represent activity of reward structures such as the orbitofrontal cortex.

In summary, the results show that pleasant and unpleasant odours are able to influence evaluations of both happy and disgusted facial expressions, and that these facial expressions are also able to modulate evaluations of odour pleasantness and intensity. It is clear that odour-face congruency has a role in these effects. Olfactory-visual interactions were represented in the N200 and N400 components of face ERPs. The effects of odour-face congruency were apparent in clean air and unpleasant odour conditions, whilst these were masked by a pleasant odour context. It is possible that the hedonic state induced by the pleasant odour was able to mask congruency effects. Congruent pairings of unpleasant odour and disgusted faces resulted in stronger shifts in face evaluation, increased odour intensity ratings and a decrease in inspiration. It is likely that the multisensory combination of congruent aversive olfactory and visual stimuli heightens withdrawal behaviours as part of an adaptive mechanism. Results also suggest that both encoding and response mechanisms are involved in cross-modal evaluative priming.

Chapter 6

Olfactory-visual integration in the frontal cortex during a hedonic rating task: an fMRI study.

This experiment investigated the effects of a pleasant odour on evaluations of flowers and objects using fMRI.

It is currently in preparation for publication for a journal to be confirmed.

The roles of the co-authors are summarised below:

I designed the study in collaboration with Andrej Stancak, and collected the data. John Tyson-Carr assisted with the data collection. Andrej Stancak and Nicholas Fallon provided expertise on the experimental set-up. Andrej Stancak and Nicholas Fallon provided training on the data analysis. John Tyson-Carr assisted with some analysis. I analysed the data, interpreted the results, and wrote the manuscript. Nicholas Fallon and Andrej Stancak provided useful comments during the preparation of the manuscript.

Acknowledgments

We gratefully acknowledge Bill Bimson, Val Adams and Martin Baker for the liberal sharing of their excellent technical expertise, and their vital support with the experimental set-up and data collection.

6.3 Abstract

Areas of the frontal cortex are known to be involved in evaluative decision making, but it is not known how subjective evaluative processes are represented in the brain in the context of a pleasant odour, and how this relates to olfactory-visual congruency. The present study aimed to investigate the neural basis of pleasant odour effects on subjective hedonic evaluations of congruent and incongruent visual stimuli, using event-related fMRI.

Twenty participants provided pleasantness ratings (value-based judgement) and colour ratings (perceptual judgement) of pictures of objects and pictures of flowers under a pleasant, floral odour condition and a no odour control condition during a single scanning session. Ratings of odour pleasantness and intensity were also recorded.

Floral odour improved subjective evaluations of all visual stimuli, whilst pictures of flowers increased pleasantness and intensity ratings of floral odour and clean air. Odour-related activations were observed in the amygdala. The superior frontal gyrus (part of the dorsomedial prefrontal cortex, dmPFC) and middle frontal gyrus were activated during hedonic evaluations, where activity in the superior frontal gyrus was boosted by a pleasant odour context. Olfactory-visual congruency effects were observed in associative brain regions and the insula. These effects were also related to subjective hedonic evaluations.

Our results support evidence for the role of the dmPFC in subjective valuation, and contribute that such activation can be boosted by a pleasant odour. Activity in associative brain regions and insula may reflect the representation of congruency and the encoding of hedonic value in the brain. A general priming effect of pleasant odour on subjective ratings of pictures, accompanied by increased activity in the amygdala during odour stimulation provides further evidence for the involvement of the amygdala in encoding hedonic valence.

6.2 Introduction

Evidence for the multisensory integration of olfactory and visual information is becoming increasingly well documented in sensory literature. Authors have argued that high level visual-olfactory cross-modal interactions are automatic (Dematte et al., 2009). Pleasant and unpleasant odours can influence hedonic evaluations of neutral or abstract images (Knasko, 1995; van Reekum et al., 1999) and human faces (Cook et al., 2015; Dematte et al., 2007; Li et al., 2007; McGlone et al., 2013). However, such olfactory-visual interactive effects on evaluations are often modulated by congruency between the odour and the visual stimulus (Bensafi, Pierson, et al., 2002; Leppanen & Hietanen, 2003; Seo, Roidl, et al., 2010). Gottfried and Dolan (2003) showed that odours were identified quicker when paired with congruent pictures. This facilitation was accompanied by enhanced activity in rostromedial orbitofrontal cortex (OFC), providing evidence for the convergence of visual and olfactory information in the OFC.

Recent studies have investigated the evidence for a valuation system in the brain that encodes subjective preferences and values of objects. Whilst animal studies have suggested that OFC encodes subjective value in non-human primates (Padoa-Schioppa, 2009; Padoa-Schioppa & Assad, 2006; Tremblay & Schultz, 1999), recent human data has suggested that the brain's valuation system includes ventromedial prefrontal cortex (vmPFC), dorsomedial prefrontal cortex (dmPFC), ventral striatum, and insula (Abitbol et al., 2015; Bartra et al., 2013; Lebreton et al., 2009). Studies have shown that value-based evaluative processes and related brain structures are activated automatically upon viewing an object; regardless of whether or not the task is to explicitly report the subjective judgement (Abitbol et al., 2015; Kühn & Gallinat, 2012; Lebreton et al., 2009; Levy et al., 2011). Further, one such study showed that a musical context influenced subjective value judgements and corresponding vmPFC activity (Abitbol et al., 2015). It is likely that an odour context would also influence subjective values and activity in the brain valuation system, but this has not yet been investigated. For example, if objects were presented with a pleasant odour, they may be evaluated more positively, particularly if congruent with the odour (Cook et al., 2015; Dematte et al., 2007; Knasko, 1995; McGlone et al., 2013).

The present study aimed to investigate how combinations of congruent visual and olfactory stimuli affect the brain valuation system during explicit, value-based judgements versus distractive, cognitive judgements of the visual stimuli. We predict that a pleasant odour will increase hedonic ratings of all visual stimuli, with an increased effect for congruent images. Pleasant odour context may mediate valuation-related activations in structures associated with the brain valuation system, such as OFC, vmPFC or dmPFC.

6.3 Methods and materials

6.3.1 Participants

A total of 20 (10 male) healthy participants aged 18−31 years (mean ± standard deviation: 23.55 ± 3.37) took part in the experiment after giving written informed consent in accordance with the Declaration of Helsinki. The study was approved by the Research Ethics Committee at the University of Liverpool. All but four participants were right handed. All participants were initially screened in a separate session using the Sniffin'Sticks (Hummel et al., 1997) test battery to ensure adequate odour identification ability. Safety screening was carried out by a research radiographer to ensure participant safety in the scanner. Participants were reimbursed for their time and travel expenses.

6.3.2 Visual and odour stimuli

Twelve pictures of flowers and twelve pictures of neutral objects were used in the experiment, for a total of 24 images. Flower pictures were selected on the basis of an unpublished study, which showed congruency effects demonstrated by reaction time when they were paired with a pleasant, floral odour (Fallon, in preparation). Object pictures were a mixture of household and leisure items, used in previously published work (Wright et al., 2015). All pictures were 492 × 330 pixels, with a consistent light background.

Odours were administered using a custom-built, continuous airflow, computer-controlled olfactometer with 8 channels (Dancer Design Ltd., UK). Odour pulses were embedded within a constant flow of clean air, in order to avoid effects of a sudden increase in airflow associated with presentation of an odour (Huart et al., 2012). Airflow was kept constant at 4 l/min. Odours and clean air flowed through two tubes situated approximately two centimetres away from the nostrils, achieved by attaching the olfactometer head piece to the scanner head coil.

There were two odour conditions in the experiment; pleasant, 'floral' odour and a neutral, 'clean air' control. The floral odour (Mistral Industrial Chemicals, Northern Ireland) was diluted at 1% in Propylene Glycol, based on perceived intensity data from a pilot study (N = 5). Propylene Glycol (1,2-Propanediol 99%, Sigma-Aldrich Ltd., UK) was used for dilution, clean air control and constant flow.

Both presentation of the experimental task stimuli and triggering of the odour valves were achieved using Cogent 2000 v. 1.32 software (Wellcome Department of Imaging Neuroscience, United Kingdom), as implemented in MATLAB 2013 (The MathWorks, Inc., USA).

6.3.3 Procedure

The experimental session lasted around two hours in total, including instructions, equipment set up, practice trials, baseline odour ratings and the experimental task. After being provided with some instructions about the experiment, participants entered the scanner room where they were asked to apply a respiratory belt and ear plugs. Once participants were comfortable on the scanner table, the head coil was adjusted to the correct position to accommodate the olfactometer head piece and tubing. Participants viewed the experimental task through a mirror reflecting a computer display projected onto a screen in the back of the scanner. Task ratings were completed using an MR compatible trackball mouse (Trackball 2, Current Designs Inc., Philadelphia, USA). Before scanning, participants completed five practice trials each consisting of two mock visual analogue scales to familiarise them with the use of the trackball. Ratings of pleasantness, intensity, and familiarity were also recorded for both the clean air and floral odour before scanning. For these

baseline ratings, four-second pulses of each were administered individually. On-screen visual analogue scales then prompted participants to rate the pleasantness (from 0 – very unpleasant to 100 – very pleasant), intensity (0 – no odour to 100 – very intense odour) and familiarity (0 – not familiar at all to 100 – extremely familiar) of the odour or clean air using the trackball mouse.

The experimental task was split into two blocks of 48 trials (96 trials in total), each lasting approximately 25 minutes. In one half of the trials, the floral odour was administered, and in the other half of trials, no odour was administered (continuous flow of clean air). In one half of the trials, flower pictures were presented, whilst in the other half of trials, object pictures were presented. Further, in one half of the trials, participants were instructed to rate the pleasantness of the item in the picture using a visual analogue scale (from 0 – very unpleasant to 100 – very pleasant). In the other half of trials, the instruction was to rate the proportion of colour of the item in the picture. For example, if the picture showed a pink and yellow flower, participants would rate closer to one end of the scale if the flower was mostly pink, or to the other end of the scale if it was mostly yellow (e.g. from 0 – pink to 100 – yellow). The colour rating served as the control rating condition. Therefore, the experiment took a 2 × 2 × 2 design with 8 conditions, where the factors were odour (floral vs clean air), picture type (flowers vs objects) and rating condition (hedonic rating vs control rating). Trials were pseudo-randomly ordered such that each of the 24 images appeared four times: twice with the floral odour/clean air and twice requiring a hedonic/perceptual evaluation.

Figure 6.1 shows a flowchart of the trial procedure. Each trial began with a 7.5 s resting interval during which participants viewed a white cross on a black background. Following this, a rating instruction (PLEASANTNESS or COLOUR) was displayed in white text for 2 s, before a red cross was displayed for a further 2 s. Participants were instructed to exhale while the red cross was displayed, in preparation to inhale (with the specific instruction not to ‘sniff’, in order to avoid percept-unrelated activations in olfactory structures, Mainland & Sobel, 2006) when a green cross was displayed for 1 s. In floral odour trials, odour onset coincided with onset of the green cross. Following this, an object or flower picture was displayed on screen for 2.5 s. Odour offset coincided with offset of the picture. Hence, odour pulses were 3.5 s in duration. Immediately after odour and picture offset, participants

were prompted to rate either the pleasantness or the colour of the object (5 s), followed by the pleasantness (from 0 – very unpleasant to 100 – very pleasant) and the intensity (0 – no odour to 100 – very intense odour) of any odour they experienced during that trial using the scales on screen (7 s).

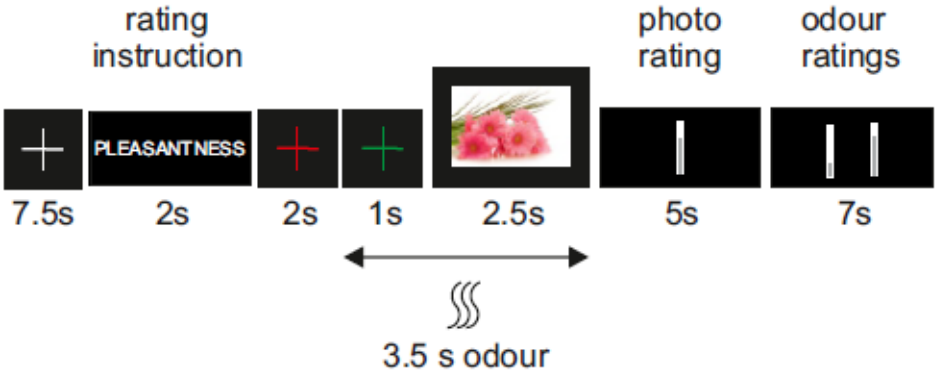

Figure 6.1: Flowchart of experimental trial procedure.

6.3.4 Image Acquisition

Scanning was carried out at the Magnetic Resonance and Image Analysis Research Centre (MARIARC) at the University of Liverpool using a whole-body Siemens Trio 3T scanner (Siemens, Erlangen, Germany) with an eight-channel radiofrequency head-coil. Foam padding and cheek clamps were used to restrict head movement. As required by MARIARC safety protocol, a clinical T2-weighted anatomical scan was acquired. This scan was not used for research purposes, but was evaluated by a qualified clinician for medical anomalies or incidental findings that would require further investigation. Following the clinical scan, a high-resolution ($1mm^3$) 3-dimensional T1-weighted scan was acquired. For the two functional scans, two dummy scans from the start of each block were discarded, in order to remove T1 saturation effects. An echo planar imaging (EPI) sequence was used to acquire functional images covering the whole brain (37 axial slices), TR = 2200 ms, TE = 30 ms, slice order = interleaved ascending, flip angle = 90°, matrix = 64 × 64, field of view = 192 mm, slice thickness = 3 mm (0.6 mm spacing), voxel size at acquisition = 3.0 × 3.0 × 3.0 mm.

6.3.5 Behavioural analyses

Ratings of odour pleasantness, intensity and familiarity taken before scanning were analysed using paired t-tests. Data from the experimental task were analysed using repeated measures ANOVAs. A 2 × 2 repeated measures ANOVA was used to analyse differences in picture pleasantness ratings depending on odour and rating condition. Picture colour ratings were not analysed as they constituted a nonsense control variable. Further, 2 × 2 × 2 repeated measures ANOVAs were used to analyse differences in odour pleasantness and intensity depending on the odour, rating, and picture condition. Significant interactions were followed up with post-hoc 2 × 2 ANOVAs and paired t-tests, using Bonferroni correction for multiple comparisons. P values in all ANOVA effects were adjusted using the Greenhouse-Geisser method. All behavioural data was analysed using SPSS v. 22 software package (IBM Inc., USA).

6.3.6 fMRI data analyses

Spatial pre-processing of functional data was performed in SPM12 running in MATLAB 2014b. Functional volumes underwent slice-timing correction, realignment, normalisation to MNI (Montreal Neuroimaging Institute) space using the normalised EPI template image in SPM and spatial smoothing (8 mm full width half maximum Gaussian kernel filter) (Friston, 2004).

First, for each participant, 660 scans per functional block were entered into a first level design including movement parameters as regressors, to define effects of condition. Scans from each condition were then combined across blocks by computing first level contrasts, resulting in 8 contrast images (1 per condition) per participant. Picture ratings were then included as covariates in this first level design and the same contrasts per subject per condition were computed.

At the second level, the first set of contrast images (no covariates) were entered into a 2 × 2 × 2 flexible factorial ANOVA including subjects as a variable (Glascher & Gitelman, 2008), to explore main effects of odour and rating condition. The main effect of picture type was not investigated, as it was beyond the scope of our hypotheses. We were only interested in effects of picture type in the context of a

congruent odour. The following contrasts were computed to explore these effects: odour > clean air, clean air > odour, hedonic rating > control rating. The contrast, control rating > hedonic rating was not investigated, as it was not relevant to the research question. In order to fully explore interactions in the effects of interest, further 2 × 2 flexible factorial ANOVAs were computed with data from the floral odour condition and hedonic rating condition, separately. For the floral odour condition, contrasts computed were: hedonic rating > control rating, flowers > objects, and interaction rating condition × picture type. In the hedonic rating condition, the following contrasts were computed: odour > clean air, flowers > objects, interaction odour × picture type.

To analyse the effects of odour, rating condition and picture type on BOLD activation that were specifically related to subjective picture ratings, contrast images that included picture ratings as covariates were entered into the three-way flexible factorial design, and the same contrasts were computed. In order to fully explore interactions also related to subjective picture ratings, contrast images from the floral odour condition including picture ratings as covariates were entered into a 2 × 2 flexible factorial design. The following contrasts were computed: hedonic rating > control rating and interaction rating condition × picture type. Further, a 2 × 2 flexible factorial ANOVA was computed with images from the hedonic rating condition including picture ratings as covariates, where the interaction between odour and picture type was investigated. These designs were identical to those discussed above, except with picture ratings included as a covariate.

A liberal threshold of uncorrected $P < 0.001$, with a minimum cluster size of 20 voxels ($k = 20$) was employed in the second level contrasts, given the exploratory nature of the research question. Significant clusters and sub-clusters were selected as regions of interest (ROIs), and defined as 5 mm diameter spheres using MNI co-ordinates in the MarsBaR 0.44 toolbox for SPM12 (http://marsbar.sourceforge.net/; Brett, Anton, Valabregue, & Poline, 2002). BOLD data for each condition for each ROI were then extracted and analysed using further 2 × 2 × 2 repeated measures ANOVAs in SPSS, with a confirmatory threshold of $P < 0.05$. Significant interactions were followed up with post-hoc 2 × 2 ANOVAs and paired t-tests.

Significant effects found in the left amygdala were followed up with further confirmatory analyses on a pre-defined small volume, using left amygdala co-ordinates from a meta-analysis by Wager, Phan, Liberzon, and Taylor (2003) (MNI

co-ordinates x = -22, y = -4, z = -14). These co-ordinates were used to create a 5 mm diameter sphere in the MarsBaR toolbox, where data was extracted and analysed further in SPSS, in line with the previous confirmatory analyses.

6.3.7 Respiratory movement signals

Participants' respiration was recorded continuously throughout the experiment with a respiratory bellows gating belt worn around the chest at the level of the epigastrium. Signals were transduced and extracted using LabChart 7 (ADInstruments Ltd., Oxford, UK). Respiratory movement signals were low-pass filtered, baseline corrected and averaged separately for each of the 8 conditions. The epochs of interest were then analysed statistically using a 2 × 2 × 2 repeated measures ANOVA in SPSS.

6.4 Results

6.4.1 Baseline odour ratings

Mean ratings of pleasantness, intensity and familiarity for both the floral odour and clean air taken before scanning are shown in Table 6.1. Paired samples t-tests confirmed that the floral odour was rated as significantly more pleasant ($t(19) = -6.49$, $P < 0.001$), and significantly more intense ($t(19) = -10.46$, $P < 0.001$) than clean air. There was no significant difference in familiarity ratings of clean air and floral odour ($P > 0.05$).

Table 6.1: Mean (± standard deviation) ratings of odour pleasantness, intensity and familiarity.

	Pleasantness	Intensity	Familiarity
Clean Air	53.7 (± 8.78)	22.35 (± 19.32)	57.1 (± 25.02)
Floral Odour	72.1 (± 9.7)	64.65 (± 13.01)	62.25 (± 16.17)

6.4.2 Picture pleasantness ratings

Figure 6.2A shows the mean pleasantness ratings of flower and object pictures under each odour condition. A 2 × 2 (odour × picture type) repeated measures ANOVA showed a significant main effect of odour on picture pleasantness ratings ($F(1, 19) = 15.89$, $P = 0.001$), confirming that all pictures were rated as more pleasant overall when they were presented with the floral odour (mean ± standard deviation, 53.65 ± 24.17) in comparison to when they were presented with clean air (45.23 ± 23.92). There was also a significant main effect of picture type on picture pleasantness ratings, confirming that pictures of flowers were rated as significantly more pleasant (69.82 ± 12.21) than pictures of objects (29.07 ± 14.57). There was no significant interaction between odour and picture type affecting picture pleasantness ratings ($P > 0.05$).

6.4.3 Odour pleasantness and intensity ratings

Figures 6.2B and 6.2C show the mean pleasantness and intensity ratings of the floral odour and clean air taken throughout the experimental task under each experimental condition, respectively. A 2 × 2 × 2 (odour × rating condition × picture type) repeated measures ANOVA revealed a significant effect of odour on odour pleasantness ratings ($P < 0.001$), confirming that the floral odour was rated as significantly more pleasant than clean air. There was also a significant main effect of picture type on odour pleasantness ratings ($P < 0.001$), revealing that both clean air and floral odour were always rated as more pleasant when paired with pictures of flowers compared to when they were paired with pictures of objects. The data showed a main effect of rating condition on odour pleasantness ratings ($P = 0.005$), suggesting that odours were rated as more pleasant when the instruction was to rate the pleasantness of the picture in that trial, compared to trials where the instruction was to rate the colour of the flower or object in the picture. Significant interactions between odour and picture type and odour and rating condition also affected odour pleasantness ratings ($P < 0.05$). There was no significant interaction between rating condition and picture type ($P > 0.05$), however, there was a significant three-way

interaction between odour, picture type and rating condition affecting odour pleasantness ratings ($P < 0.05$).

To further investigate the interactions of interest, post-hoc 2 × 2 (rating condition × picture type) ANOVAs were computed on pleasantness ratings for the floral odour and clean air conditions separately. These revealed a significant main effect of rating type affecting pleasantness ratings of the floral odour ($P < 0.001$), suggesting that it was always rated as more pleasant when the instruction was to rate the pleasantness of the flower or object in the picture in comparison to when the instruction was to rate the colour of the flower or object. The data also showed a significant main effect of picture type on pleasantness ratings of the floral odour ($P < 0.001$), suggesting that the floral odour was always rated as more pleasant when paired with pictures of flowers in comparison to when it was paired with pictures of objects. A significant interaction between picture type and rating condition ($P = 0.004$) and post hoc t-tests indicated that when the floral odour was paired with pictures of flowers, it was rated as more pleasant when the trial instruction was to rate the pleasantness of the flowers compared to when the instruction was to rate the colour of the flowers ($P < 0.001$). When the floral odour was paired with pictures of objects, there was no significant difference in odour pleasantness ratings between rating conditions ($P > 0.05$).

There was a significant main effect of picture type on pleasantness ratings of clean air ($P = 0.04$), suggesting that clean air was rated as significantly more pleasant when it was paired with pictures of flowers compared to when it was paired with pictures of objects. However, there was no significant effect of rating condition ($P > 0.05$), nor a significant interaction between rating condition and picture type affecting pleasantness ratings of clean air ($P > 0.05$). Odour intensity ratings showed the exact same pattern of effects as odour pleasantness ratings (see Figures 6.2B & 6.2C).

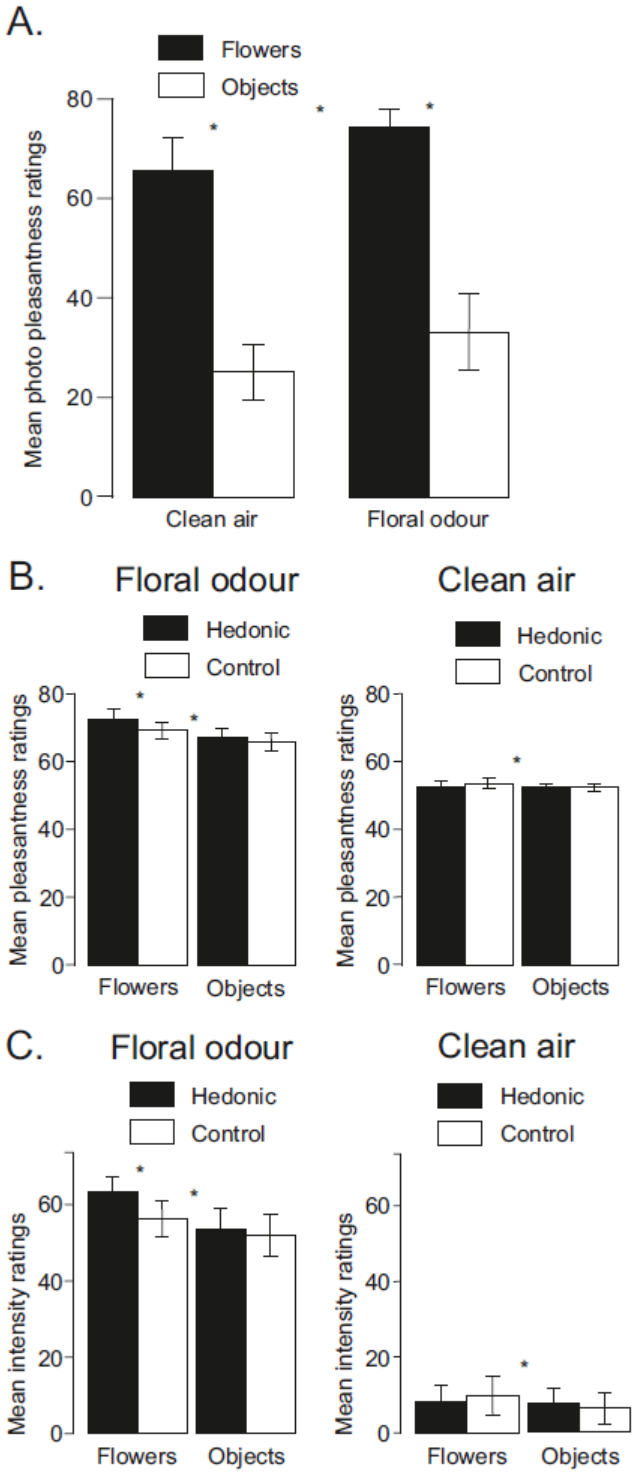

Figure 6.2: Behavioural data. (A) Mean picture pleasantness ratings under each condition. Black bars represent ratings of pictures of flowers, and white bars represent mean ratings of object pictures. Error bars represent 95% confidence intervals. Asterisks highlight significant differences between flower and object pictures ($P < 0.05$, Bonferroni corrected). (B) Mean odour pleasantness ratings under all experimental conditions. Black bars represent ratings from trials that required a hedonic rating of the picture; white bars represent trials that required participants to rate the colour of the picture (control). Error bars represent 95% confidence intervals. Asterisks highlight significant differences between flower and object pictures and hedonic and control rating conditions ($P < 0.05$, Bonferroni corrected). (C) Mean odour intensity ratings under all experimental conditions. Specifications are identical to (B).

6.4.4 fMRI data

We first present whole-brain analyses investigating effects of odour and hedonic rating. To explore how such effects were specifically related to subjective hedonic ratings of pictures, we then report whole brain analyses from the conditions of interest, using picture ratings as a covariate. Given that a liberal, uncorrected threshold of $P < 0.001$ was employed, whole brain results must be considered exploratory.

6.4.4.1 Whole brain analyses without covariates

A 2 × 2 × 2 ANOVA across all data revealed a significant main effect of odour (uncorrected $P < 0.001$). The contrast odour > clean air revealed 2 significant clusters in the left amygdala and right parahippocampal gyrus (see Table 6.2 and Figure 6.3). Significant clusters were defined as ROIs from which data was extracted and further analysed. For each ROI, Tables 6.2−6.7 list the anatomical location, MNI co-ordinates, hemisphere, T value, cluster size and Brodmann area, along with F values and P values for the statistical effects. All main effects are discussed. Some interaction effects are not discussed as they were beyond the scope of the research question. Further analyses revealed greater activation in both the amygdala and parahippocampal gyrus in the floral odour condition compared to the clean air condition. There was also stronger activation in the amygdala during trials showing pictures of flowers compared to those showing pictures of objects (see Figure 6.3). There were no significant clusters in the contrast, clean air > odour.

To further confirm effects in the amygdala, we analysed whether the findings remained significant with correction in a pre-defined small volume. Analysis on the pre-defined amygdala ROI showed significantly greater activation in the floral odour condition compared to clean air ($P = 0.002$), greater activation during hedonic rating compared to control ($P = 0.02$), and marginally stronger activation during trials showing pictures of flowers compared to those showing pictures of objects ($P = 0.07$).

The three-way ANOVA also showed a significant main effect of rating condition ($P < 0.001$). The contrast, hedonic rating > control rating, yielded three significant clusters, each with multiple sub-clusters. The clusters were located in the superior frontal gyrus, posterior cingulate and cingulate gyrus, and the middle temporal gyrus (see Table 6.2). Confirmatory analysis revealed stronger activation in the hedonic rating condition compared to control condition across all three clusters and sub-clusters.

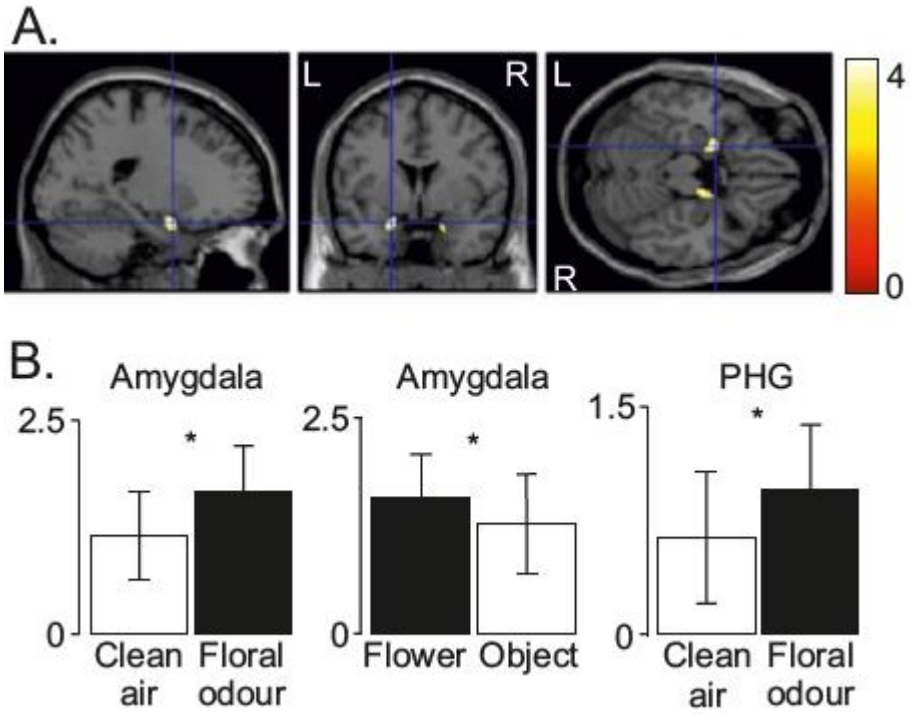

Figure 6.3: (A) A contrast revealing the effect of odour (odour > clean air) in the left amygdala and right parahippocampal gyrus (PHG), which were then defined as ROIs (co-ordinates and values are shown in Table 6.2). 'L' and 'R' represent left and right hemisphere, respectively. (B) Bar graphs showing significant effects of experimental condition on mean BOLD activity in ROIs. Error bars represent 95% confidence intervals. Asterisks highlight significant differences between clean air and floral odour conditions and flower and object pictures ($P < 0.05$).

To further investigate interactions between rating condition and picture type in the presence of a pleasant odour, a 2 × 2 ANOVA on data from the floral odour condition only was computed, revealing a main effect of picture type ($P < 0.001$). The contrast flowers > objects revealed two large clusters, with sub-clusters, in the lingual gyrus, inferior occipital gyrus, claustrum and insula (see Table 6.3 and Figure

6.4). All clusters and sub-clusters showed greater activation to pictures of flowers compared to pictures of objects.

There was also a significant main effect of rating condition ($P < 0.001$) on data from the floral odour condition. The contrast hedonic rating > control rating revealed four significant clusters in the superior frontal gyrus and the posterior cingulate (see Table 6.3), all showing stronger activation in response to hedonic rating trials in comparison to control rating trials. There was no significant interaction between rating condition and picture type ($P > 0.001$).

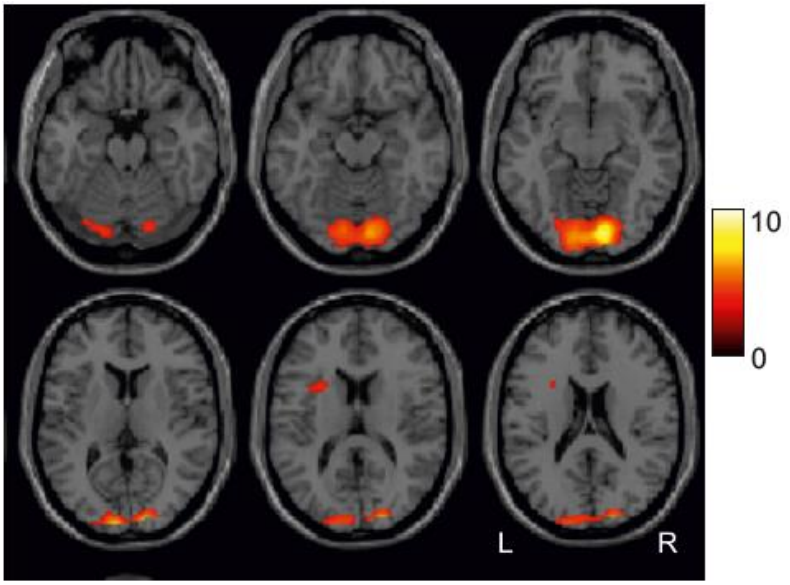

Figure 6.4: Axial slices from a contrast highlighting the effect of picture type in the floral odour condition only (flowers > objects). Significant clusters were revealed in the lingual gyrus, inferior occipital gyrus, claustrum and insula (co-ordinates and values are shown in Table 6.3). 'L' and 'R' represent left and right hemisphere, respectively.

In order to examine interactions between odours and pictures in the hedonic rating condition specifically, a 2 × 2 ANOVA on data from the hedonic rating condition was employed. There was no main effect of odour ($P > 0.001$); however, there was an effect of picture type ($P < 0.001$). The contrast flowers > objects showed 8 significant clusters, with some sub-clusters, including the lingual gyrus, cuneus, amygdala, inferior parietal lobule, supramarginal gyrus, superior temporal gyrus and insula (see Table 6.4 and Figure 6.5). Confirmatory analyses revealed greater activation to pictures of flowers in comparison to pictures of objects across

all 8 clusters. Further analysis on the pre-defined amygdala volume confirmed that in the hedonic rating condition, there was significantly greater activation in trials showing pictures of flowers compared to pictures of objects (P = 0.03).

A significant interaction between odour and picture type in the hedonic rating condition (P < 0.001) revealed a significant cluster in the inferior temporal gyrus. Further analysis confirmed a significant interaction between odour and picture type (see Table 6.4). Post-hoc t-tests were employed to further investigate this interaction and showed a stronger activation to pictures of objects compared to pictures of flowers in the floral odour condition (t(19) = -3.56, P = 0.002), and greater activation to pictures of flowers compared to objects in the clean air condition (t(19) = 2.45, P = 0.02).

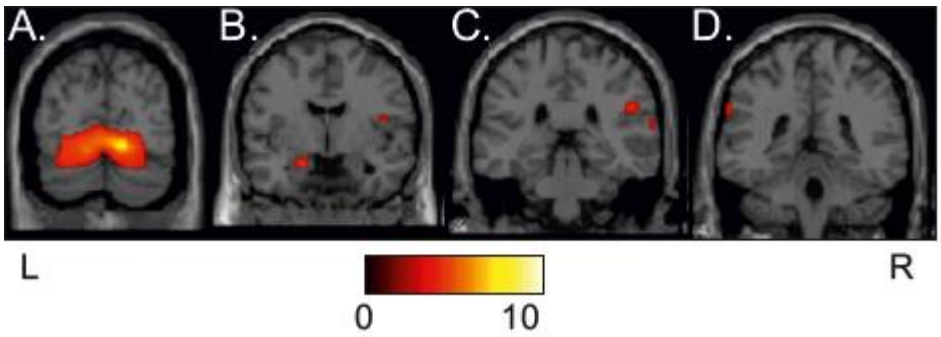

Figure 6.5: Coronal slices from a contrast showing the effect of picture type in the hedonic rating condition only (flowers > objects). Significant clusters were revealed in (A) lingual gyrus, cuneus, (B) amygdala, insula, (C) inferior parietal lobule, superior temporal gyrus and (D) supramarginal gyrus (co-ordinates and values are shown in Table 6.4). 'L' and 'R' represent left and right hemisphere, respectively.

6.4.4.2 Whole brain analyses using picture ratings as a covariate

Picture ratings were included as a covariate to further investigate effects that were specifically related to subjective picture pleasantness ratings. In the 2 × 2 × 2 ANOVA, there was an effect of rating condition, where the contrast hedonic rating > control rating revealed four significant clusters, located in the cerebellum, middle frontal gyrus, the inferior parietal lobule and the superior frontal gyrus (see Figure 6.6). Table 6.5 lists spatial information and statistics for each cluster and their sub-

clusters. Confirmatory analysis revealed a significant effect of rating condition across all clusters, showing stronger activations in the hedonic rating condition compared to the control rating condition that were also related to picture pleasantness ratings.

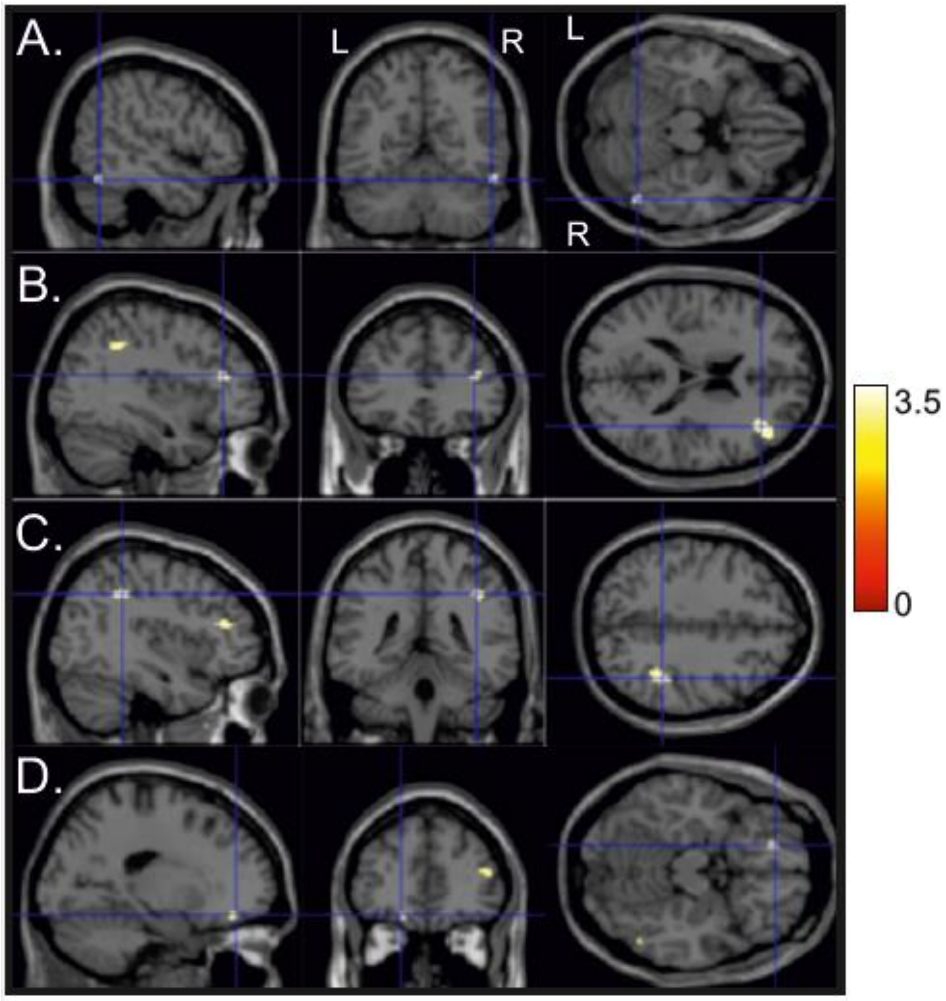

Figure 6.6: A contrast revealing the effect of rating condition (hedonic rating > control) where picture ratings were included as a covariate revealed clusters in (A) cerebellum, (B) middle frontal gyrus, (C) inferior parietal lobule, (D) superior frontal gyrus, which were defined as ROIs (co-ordinates and values are shown in Table 6.5). Confirmatory analysis showed a significant effect of rating condition on BOLD signal (hedonic rating > control) in all regions. 'L' and 'R' represent left and right hemisphere, respectively.

In the floral odour condition, there was a significant main effect of rating condition, with the contrast hedonic rating > control rating revealing significant activation in the superior frontal gyrus (see Figure 6.7 and Table 6.6) that was also related to picture pleasantness ratings. Further analysis confirmed greater activation for hedonic rating compared to control. There was no significant main effect of picture type related to picture ratings in the floral odour condition ($P > 0.001$).

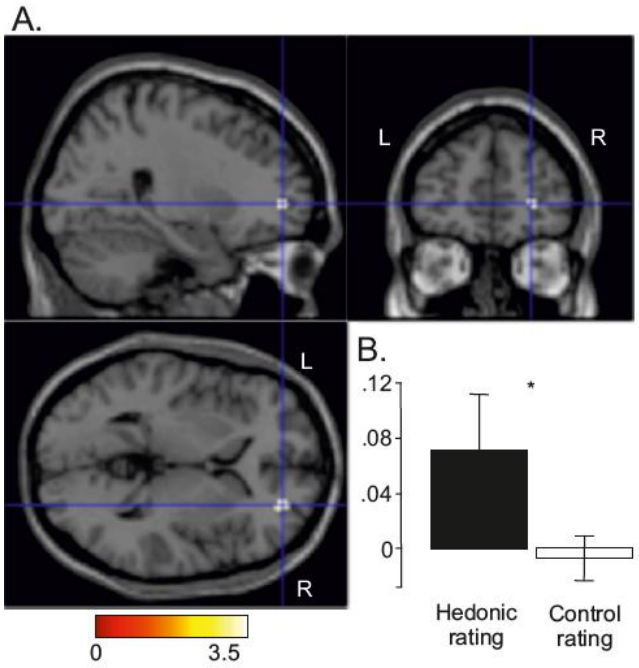

Figure 6.7: (A) A contrast showing an effect of rating condition (hedonic rating > control) in the superior frontal gyrus in the floral odour condition, that was also related to picture pleasantness ratings. (B) Bar graph showing the mean BOLD activation from this ROI, where the black bar represents the mean from hedonic rating trials, and the white bar represents the mean from control rating trials. Error bars show 95% confidence intervals. 'L' and 'R' represent left and right hemisphere, respectively. An asterisk indicates the significant difference between the hedonic rating and control rating condition ($P < 0.05$).

When picture ratings were included as a covariate in a 2 × 2 ANOVA on data from the hedonic rating condition, there were no significant effects of odour or picture type ($P > 0.001$). However, there was a significant interaction between odour and picture type related to subjective picture pleasantness ratings. The contrast investigating this interaction revealed a large cluster encompassing three sub-clusters in the parahippocampal gyrus, middle temporal gyrus and insula (see Table 6.7 and Figure 6.8). Confirmatory analysis revealed a significant interaction between odour and picture type across all sub-clusters. Post-hoc t-tests revealed that in the insula, there was a greater activation to objects compared to flowers in the floral odour condition ($P = 0.01$), and greater activation to flowers compared to objects in the clean air condition ($P = 0.01$). In the parahippocampal gyrus, there was a stronger activation towards objects compared to flowers in the floral odour condition ($P = 0.001$), and a trend towards stronger activation to flowers compared to objects in the clean air condition ($P = 0.03$). In the middle temporal gyrus, there was a stronger

activation to objects compared to flowers, in the floral odour condition only (P = 0.004).

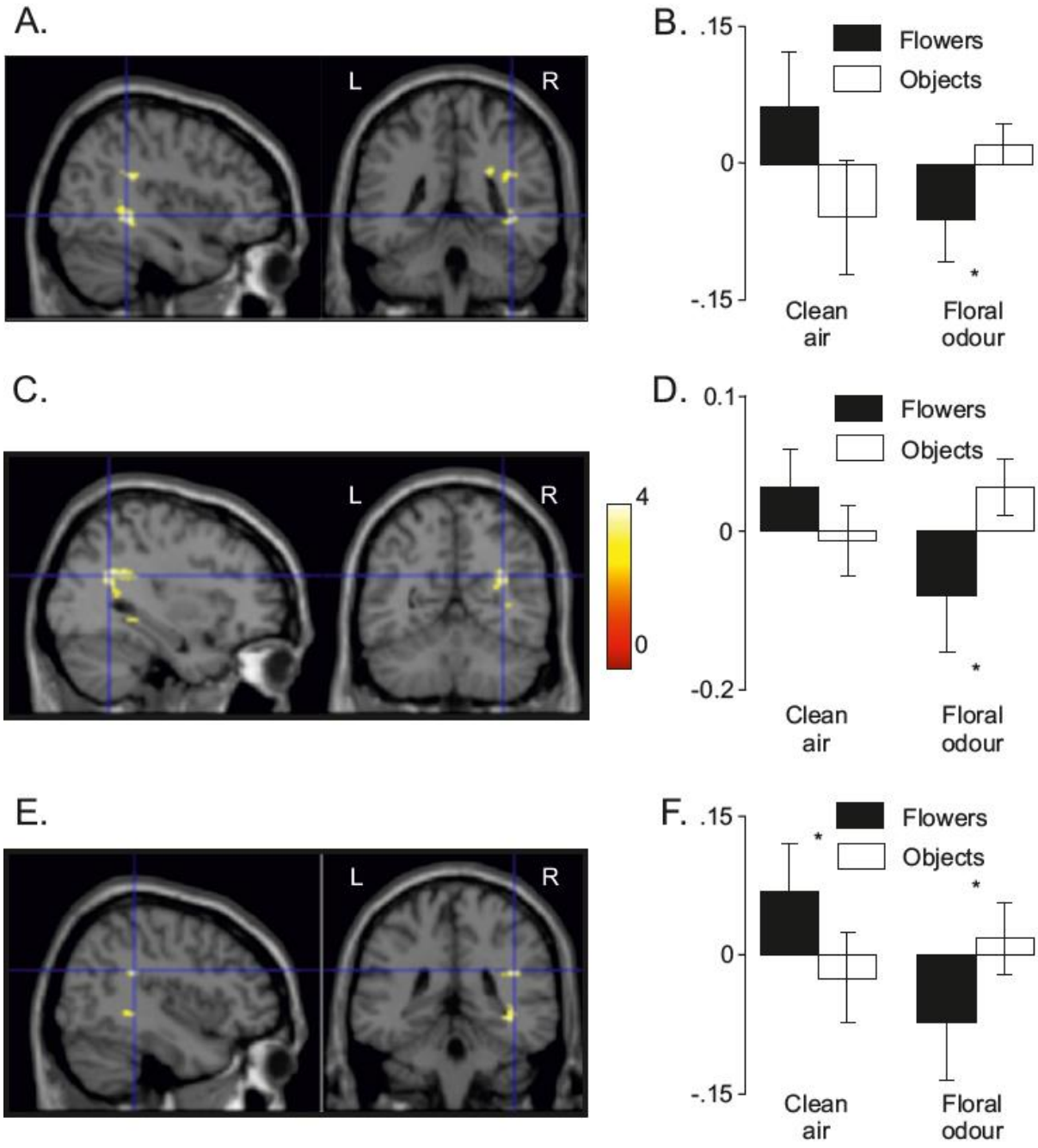

Figure 6.8: A contrast showing the interaction between odour and picture type in the hedonic rating condition only, including picture pleasantness ratings as a covariate revealed significant clusters in (A) parahippocampal gyrus, (B) middle temporal gyrus and (C) insula. (B) Bar graph showing the mean BOLD activation under each odour and picture condition in the parahippocampal gyrus. Black bars represent trials showing pictures of flowers, and white bars represent trials showing phots of objects. Error bars show 95% confidence intervals. Significant differences ($P < 0.025$) between flower and object picture conditions are highlighted using asterisks. (D) Bar graph showing the mean BOLD activation under each odour and picture condition in the middle temporal gyrus. (F) Bar graph showing the mean BOLD activation under each odour and picture condition in the insula. 'L' and 'R' represent left and right hemisphere, respectively.

6.4.5 Respiratory movement analysis

Figure 6.9A shows averaged respiratory waveforms for each condition. Mean respiratory movement values from an epoch spanning the inspiratory cycle, beginning at odour onset (2−5 s) were analysed in a 2 × 2 × 2 ANOVA. This revealed significant main effects of odour ($P < 0.001$), rating condition ($P = 0.04$), and picture ($P < 0.001$), suggesting greater inspiration during floral odour, hedonic rating and flower picture conditions, respectively. There were also significant interactions between odour and picture ($P = 0.03$), rating and picture ($P < 0.001$), and a three-way interaction between odour, rating and picture ($P < 0.001$) affecting respiratory movements. Post-hoc 2 × 2 ANOVAs and t-tests showed that in the floral odour condition, there was greater inspiration during trials requiring hedonic ratings of objects compared to control ($P = 0.001$), and no difference in inspiration between rating conditions in flower picture trials ($P > 0.05$) (see Figure 6.9B). In the clean air condition, there was smaller inspiration in trials instructing hedonic ratings of flowers compared to control ($P < 0.001$), and greater inspiration for trials requiring hedonic ratings of objects compared to control ($P < 0.001$) (see Figure 6.9B). Data from the expiratory epoch showed similar interactions; however, these are not reported as they occurred after the time of olfactory-visual stimulation and were therefore beyond the scope of the present study.

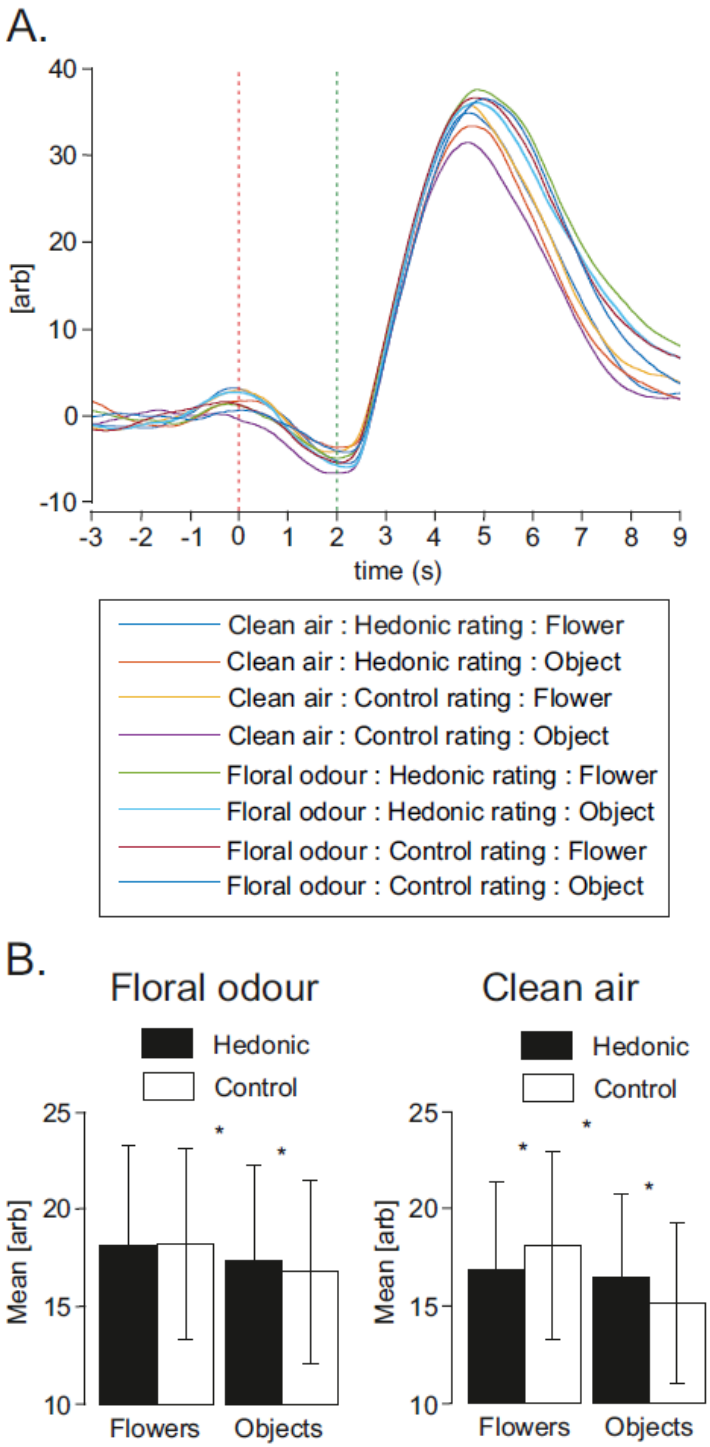

Figure 6.9: (A) Averaged respiratory waveforms for each condition. Respiratory movement signals from every subject across all trials were averaged over a period of 12 seconds. The baseline period ranged from -3 to 0 s. The red dotted line represents the 'get ready' prompt (red cross, 0 s), and the green dotted line represents odour onset and the prompt to breathe in (green cross, 2 s). Odour offset was at 5.5 s. Upwards deflection of respiratory signals corresponds to inspiration. (B) Mean inspiratory movement values (2−5 s) under each experimental condition. Black bars represent trials that required a hedonic rating of the picture; white bars represent trials that required participants to rate the colour of the picture (control). Asterisks highlight significant differences between flower and object picture conditions, and hedonic and control rating conditions ($P < 0.025$).

Table 6.2: 2 × 2 × 2 ANOVA across all data, no covariates

Anatomical location	MNI x, y, z	Hemisphere	Cluster size (k)	Brodmann area	Effect	F value	P value
Amygdala	-20, 0, -20	Left	66	-	Odour > clean air Flowers > objects	33.98 4.96	< 0.001 0.04
Parahippocampal gyrus	14, -8, 18	Right	39	-	Odour > clean air	9.49	0.006
Superior frontal gyrus	20, 38, 44	Right	1669	8	Hedonic rating > control	17.78	< 0.001
Superior frontal gyrus	-18 30 48	Left	-	8	Hedonic rating > control	11.93	0.003
Superior frontal gyrus	6 58 24	Right	-	9	Hedonic rating > control	13.21	0.002
Posterior cingulate	6 -50 24	Right	934	23	Hedonic rating > control	29.65	< 0.001
Cingulate gyrus	-10 -52 26	Left	-	31	Hedonic rating > control	14.72	0.001
Middle temporal gyrus	-62 -42 2	Left	402	21	Hedonic rating > control Odour × rating	25.16 7.32	< 0.001 0.014
Middle temporal gyrus	-54 -40 -2	Left	-	21	Hedonic rating > control Odour × rating	37.81 9.14	< 0.001 0.007
Middle temporal gyrus	-58 -32 -2	Left	-	-	Hedonic rating > control Odour × rating Odour × photo	20.33 11.58 5.0	< 0.001 0.003 0.038

Table 6.3: 2 × 2 ANOVA on floral odour condition, no covariates

Anatomical location	MNI x, y, z	Hemisphere	Cluster size (k)	Brodmann area	Effect	F value	P value
Superior frontal gyrus	-20 34 50	Left	73	8	Hedonic rating > control	9.17	0.007
Superior frontal gyrus	-12 14 70	Left	26	6	Hedonic rating > control	6.49	0.02
Superior frontal gyrus	18 44 46	Right	26	8	Hedonic rating > control	6.72	0.02
Posterior cingulate	4 -50 24	Right	67	23	Hedonic rating > control	24.9	< 0.001
Lingual gyrus	-10 -94 0	Left	3175	17	Flowers > objects	67.95	< 0.001
Lingual gyrus	14 -92 0	Right	-	17	Flowers > objects	91.58	< 0.001
Inferior occipital gyrus	12 -88 -8	Right	-	17	Flowers > objects	90.07	< 0.001
Claustrum	-26 12 18	Left	65	-	Flowers > objects	10.12	0.005
Insula	-36 6 14	Left	-	13	Flowers > objects	6.93	0.016

Table 6.4: 2 × 2 ANOVA on hedonic rating condition, no covariates

Anatomical location	MNI x, y, z	Hemisphere	Cluster size (k)	Brodmann area	Effect	F value	P value
Lingual gyrus	-4 -90 -6	Left	5338	17	Flowers > objects	130.72	< 0.001
Lingual gyrus	10 -86 -10	Right	-	18	Flowers > objects	97.66	< 0.001
Cuneus	20 -90 18	Right	-	18	Flowers > objects	29.96	< 0.001
Amygdala	-18 -4 -22	Left	34	-	Flowers > objects	19.12	< 0.001
Inferior parietal lobule	52 -32 28	Right	56	40	Flowers > objects	11.65	0.003
Supramarginal gyrus	-62 -42 30	Left	45	40	Flowers > objects Odour × photo	18.17 5.12	< 0.001 0.04
Superior temporal gyrus	68 -30 14	Right	20	42	Flowers > objects	11.42	0.003
Insula	44 -4 16	Right	41	13	Flowers > objects	26.61	< 0.001
Inferior temporal gyrus	-56 -32 -16	Left	179	20	Odour × photo	10.39	0.004

Table 6.5: 2 × 2 × 2 ANOVA with picture ratings as a covariate

Anatomical location	MNI x, y, z	Hemisphere	Cluster size (k)	Brodmann area	Effect	F value	P value
Cerebellum	52 -58 -20	Right	20	-	Hedonic rating > control	15.43	0.001
Middle frontal gyrus	38 34 18	Right	108	46	Hedonic rating > control	9.42	0.006
Middle frontal gyrus	46 48 12	Right	-	10	Hedonic rating > control	11.74	0.003
Inferior parietal lobule	40 -40 40	Right	73	40	Hedonic rating > control	10.39	0.004
Superior frontal gyrus	-18 42 -16	Left	20	11	Hedonic rating > control	14.09	0.001

Table 6.6: 2 × 2 ANOVA on floral odour condition with picture ratings as a covariate

Anatomical location	MNI x, y, z	Hemisphere	Cluster size (k)	Brodmann area	Effect	F value	P value
Superior frontal gyrus	26 50 2	Right	27	-	Hedonic rating > control	10.55	0.004
Anterior cingulate	6 18 18	Right	170	33	Objects > flowers Rating × photo	5.37 15.95	0.032 0.001
Caudate	-8 16 18	Left	-	-	Objects > flowers Rating × photo	6.63 14.05	0.02 0.001
Caudate	0 4 12	-	-	-	Rating × photo	16.11	0.001
Superior temporal gyrus	30 -52 24	Right	195	39	Objects > flowers Rating × photo	16.61 9.48	0.001 0.006
Insula	36 -44 24	Right		13	Objects > flowers Rating × photo	6.64 12.13	0.018 0.002
Middle occipital gyrus	22 -84 12	Right	136	18	Rating × photo	10.19	0.005
Lingual gyrus	22 -90 -2	Right	-	17	Rating × photo	6.94	0.02
Middle occipital gyrus	32 -84 10	Right	-	19	Rating × photo	8.41	0.009

Table 6.7: 2 × 2 ANOVA on hedonic rating condition with picture ratings as a covariate

Anatomical location	MNI x, y, z	Hemisphere	Cluster size (k)	Brodmann area	Effect	F value	P value
Parahippocampal gyrus	40 -42 -2	Right	346	19	Odour × photo	15.96	0.001
Middle temporal gyrus	34 -56 24	Right	-	39	Odour × photo	11.06	0.004
Insula	42 -38 26	Right	-	13	Odour × photo	12.3	0.002

6.5 Discussion

Pleasant odour and visual stimuli exerted bidirectional cross-modal effects on subjective ratings of visual stimuli, odour perception and respiratory patterns. Floral odour increased subjective pleasantness ratings of pictures of flowers and objects, whilst odour and clean air were rated as more pleasant and more intense when paired with pictures of flowers. The amplitude of participants' respiratory movements was greater during trials showing pictures of flowers. The key finding refers to activations in the superior frontal gyrus and other areas of frontal cortex that were specific to the hedonic rating task, and related to subjective ratings. In particular, activation in the superior frontal gyrus during hedonic ratings primed by a pleasant odour was related to subjective ratings.

As hypothesised, all pictures were rated as more pleasant when paired with the floral odour, suggesting a pleasant odour-priming effect on perception of visual stimuli, consistent with several previous studies (Cook et al., 2015; Dematte et al., 2007; Knasko, 1995; McGlone et al., 2013; Seubert et al., 2014). In addition to the effect of pleasant odour on subjective hedonic ratings of visual stimuli, pictures of flowers also resulted in greater pleasantness and intensity ratings of both pleasant odour and clean air. As observed in some previous studies (Cook et al., in preparation; Pollatos et al., 2007; Seo, Arshamian, et al., 2010), this also shows a general priming effect of visual stimuli on odour perception. Further, the floral odour was rated as more pleasant and more intense when the experimental trial required a hedonic rating of a flower picture compared to control.

6.5.1 fMRI findings

Stronger activation in response to pleasant odour compared to clean air was observed in the left amygdala and right parahippocampal gyrus. The amygdala effect remained significant when tested in a pre-defined small volume. The amygdala is implicated in basic perception of odours as well as higher order affect-related odour processing (Anderson et al., 2003; Gottfried, Deichmann, et al., 2002; Mackay-Sim & Royet, 2006; Royet et al., 2000; Zald & Pardo, 1997; Zald & Pardo, 2000). The

parahippocampal gyrus is in close proximity with primary olfactory areas in the limbic lobe, both anatomically and functionally (Squire & Zola-Morgan, 1991; Van Hoesen, Augustinack, Dierking, Redman, & Thangavel, 2000). Our data therefore support the role of the amygdala and parahippocampal gyrus in olfactory perception. We did not find any odour-related activation in the piriform cortex. Although the piriform is known as the key part of the primary olfactory system, activation has been inconsistent across studies (Zald & Pardo, 2000).

The key finding pertains to stronger BOLD activity in the superior frontal gyrus and middle frontal gyrus during the hedonic rating task that was also related to subjective hedonic ratings. Within this effect, activation in the superior frontal gyrus was specifically affected by the pleasant odour context. Significant clusters in the middle frontal gyrus and superior frontal gyrus were located in Brodmann areas 10 and 11, respectively, which are in close relation to the OFC (Elliott, Dolan, & Frith, 2000). The superior frontal gyrus also overlaps with areas described as dmPFC (Bartra et al., 2013; Petrides & Pandya, 2012). Our results therefore support the role of specific regions in the frontal cortex in encoding preferences and hedonic value of objects (Bartra et al., 2013; Lebreton et al., 2009). Taking into consideration the covariance with subjective picture ratings, the findings further support studies showing that activations in dmPFC correlate with subjective emotional experience and pleasantness ratings of affective stimuli or rewards (Bartra et al., 2013; Lebreton et al., 2009), and therefore support the role of this region as part of the brain's valuation system. Moreover, the increase in hedonic ratings of visual stimuli and corresponding activation in the superior frontal gyrus in the presence of a pleasant odour resembles findings from previous work showing that musical context influenced both subjective value judgements and activity in the vmPFC in the brain valuation system (Abitbol et al., 2015). Our results support and extend these findings, demonstrating that activity in the frontal cortex is related to subjective evaluative processes, and can be altered by olfactory context.

The cerebellum also showed a stronger response in the hedonic rating task that was related to subjective ratings. Although traditionally associated with motor function, more recently it has become accepted that the cerebellum is also involved in cognitive functions (M. Ito, 1993). It has been suggested that the cerebellum encodes models that reproduce properties of mental representations in cerebral cortex

(M. Ito, 2008), therefore it may have had a role in higher order cognitive processing during hedonic evaluations.

An interaction between odour and picture type that was also related to subjective ratings of pictures was observed in the parahippocampal gyrus, middle temporal gyrus and insula. The interactions included stronger activation in response to pictures of objects in the floral odour condition in all regions, and stronger activation to pictures of flowers in the clean air condition in the insula. The insula is implicated in a wide range of cognitive and emotional activity, given its proximity to the limbic system (Cloutman, Binney, Drakesmith, Parker, & Lambon Ralph, 2012), and agranular insula is known to be preferentially activated during higher order tasks involving odours, including explicit hedonic judgements (Royet et al., 2003; Royet et al., 2000). The insula has also been consistently named as part of the brain's valuation system (Bartra et al., 2013). Insula activation and corresponding subjective ratings in the present study therefore provide evidence for the involvement of the insula in subjective valuation, and suggest that this effect may be influenced by the presence of an odour. The insula is also associated with encoding saliency (Bartra et al., 2013; Menon & Uddin, 2010). Theories of salience suggest that stimuli are salient when they appear to be congruent or incongruent in a certain context (Guido, 2001). Hence, activation of the insula during olfactory-visual stimulation may be representative of congruency effects, as well as subjective valuation. Moreover, the parahippocampal gyrus is closely related to olfactory cortices and their function (Van Hoesen et al., 2000), and is involved in the processing of contextual associations (Aminoff, Kveraga, & Bar, 2013). The middle temporal gyrus has been implicated in semantic tasks (Vandenberghe, Price, Wise, Josephs, & Frackowiak, 1996; Whitney, Kirk, O'Sullivan, Lambon Ralph, & Jefferies, 2010). Activation in the parahippocampal gyrus and middle temporal gyrus in the present study may also reflect olfactory-visual congruency effects.

In the floral odour condition specifically, there was a greater BOLD response to pictures of flowers compared to pictures of objects in other regions including the insula, lingual gyrus, inferior occipital gyrus, and claustrum. Activity in these regions may be characteristic of the congruency between the floral odour and pictures of flowers. The lingual gyrus and inferior occipital gyrus both form part of the visual cortex, known to be involved in face and object processing, and visual association

(Macaluso, Frith, & Driver, 2000; Sergent, Ohta, & MacDonald, 1992; Zeki et al., 1991). As mentioned above, the insula has been implicated in the processing of salience, and may respond to congruency (Menon & Uddin, 2010). Hence, activation in the lingual gyrus and insula may be representative of congruency effects. Greater response to pictures of flowers also appeared in the hedonic rating condition in the insula, lingual gyrus, amygdala, inferior parietal lobule, supramarginal gyrus, superior temporal gyrus and cuneus. Activation in these areas may further represent congruency between the hedonic rating condition and pictures of flowers, given that flowers were consistently rated as more pleasant than objects.

We observed stronger amygdala activation in response to pictures of flowers compared to pictures of objects, specifically in the hedonic rating condition. This effect was also significant in the pre-defined amygdala ROI. Moreover, analysis in the pre-defined amygdala volume showed significantly greater activation to floral odour, and during the hedonic rating condition. The amygdala responds to both positive and negative stimuli, and has been reported to encode subjective valence (T. Ball et al., 2009; Jin et al., 2015; Phelps & LeDoux, 2005; Zald, 2003). Given the greater activation in the hedonic rating condition, and that pictures of flowers were rated as more pleasant than pictures of objects, amygdala activation in the present study may be representative of valence encoding, as well as basic odour perception.

6.5.2 Behavioural interactions

Behavioural data showed that the floral odour was rated as more pleasant and more intense when the experimental trial required a hedonic rating of a flower picture compared to control. This suggests a congruency effect between floral odour and pictures of flowers, but also indicates that this effect was influenced by the affective focus in the hedonic rating condition. For instance, in trials where picture pleasantness ratings were required, participants may have increased their focus towards hedonic ratings, resulting in increased odour pleasantness ratings. This finding is somewhat consistent with a study showing that participants responded to odours differently when instructed to focus on the affective value of the odour, demonstrated by greater activations in inferior frontal gyrus (Rolls et al., 2008).

Authors suggested that whether cognitive demand is affect-related versus sensory-related may be an important aspect of cognition (Rolls et al., 2008). This does not explain the additional increase in odour intensity ratings; however, although valence and intensity can be dissociated in odour perception, their ratings often correlate (Anderson et al., 2003; Doty, 1975). Therefore, affective related focus may have contributed to the increase in both pleasantness and intensity ratings during the hedonic rating condition.

6.5.3 Respiratory patterns

Results showed increased odour pleasantness and intensity ratings and increased amplitude of respiratory movements during trials showing pictures of flowers. The boost in odour pleasantness and intensity ratings may be attributable to the inspiratory increase. However, this effect occurred in both floral odour and no odour conditions, suggesting an automatic priming effect of pictures of flowers on subjective odour perception and respiratory patterns, rather than an effect of congruency between the floral odour and pictures of flowers. We therefore argue that visual stimuli produced genuine priming effects on pleasantness and intensity perception during odour stimulation and a clean air control, where increased inspiration was a by-product of such effects.

6.5.4 Limitations

With the exception of the amygdala results confirmed in a pre-defined small volume, our findings must be considered exploratory due to the use of a threshold uncorrected for multiple comparisons. We demonstrated effects of a hedonic rating task in regions in close relation to the OFC that corresponded with subjective hedonic ratings primed by a pleasant odour. However, we did not find effects of odour or olfactory-visual integration in the OFC specifically. The OFC is among the most consistently activated structures in olfactory imaging experiments (Sobel et al., 2000; Zald & Pardo, 1997; Zatorre et al., 2000), and is thought to be involved in higher-order affective processes representing the convergence of olfactory and visual

stimuli (Gottfried, O'Doherty, et al., 2002). OFC is affected by susceptibility gradients in echo planar imaging, which may have resulted in image distortions and signal loss (Deichmann, Gottfried, Hutton, & Turner, 2003). Hence, the scanning parameters used in the present study may not have been adequate to pick up effects of odours or olfactory-visual integration in the OFC (at least not in a whole-brain analysis), and we accept this as a limitation. Further, not all effects and interactions in ROIs have been discussed, as many were beyond the scope of this paper.

6.5.5 Summary

In summary, the present study showed increased activation in the superior frontal gyrus and middle frontal gyrus during a hedonic rating task that was specifically related to subjective ratings. Such activity in the superior frontal gyrus was influenced by a pleasant odour context. Our results therefore support the role of the frontal cortex in evaluative processes specific to subjective ratings, and show that the related neural activity may be further boosted by a pleasant odour. Results showed bidirectional olfactory-visual priming effects of a pleasant odour on subjective ratings of pictures, and of flower pictures on odour pleasantness and intensity ratings and respiratory patterns. Increased activity in the amygdala during odour stimulation and during hedonic ratings of pictures of flowers provides further evidence for its involvement in encoding valence. We also observed congruency effects in associative brain regions and the insula, which were related to subjective hedonic ratings. Our data provide insight into the representation of congruency and the encoding of hedonic value in the brain, and contribute to the understanding of how odours may influence these processes.

Chapter 7

General Discussion

The overall aim of this thesis was to explore the effects of odours on hedonic evaluations, and to shed light on the neural mechanisms underlying such effects using EEG and fMRI. We hypothesised that pleasant and unpleasant odours would modulate hedonic evaluations of neutral, or congruent and incongruent visual stimuli, and that such modulations would vary as a function of temporal association and rating task. It was anticipated that these effects would be related to changes in visual ERPs and activations in the brain's valuation system.

7.1 Summary of findings

- Pleasant and unpleasant odours were able to prime hedonic ratings of human faces, flowers and objects, both with and without a temporal lag between olfactory-visual presentations. Pleasant odours increased hedonic ratings of visual stimuli, whilst unpleasant odours decreased such ratings.
- Happy and disgusted faces presented with congruent pleasant and unpleasant odours were rated more or less pleasant, respectively.
- Effects of odours on the ERP response to faces were observed in the N170, mid components (N200, N400), late- and ultra-late ERP components.
- In the ultra-late ERP component (around 900 ms), activations were greater in the left and right hemispheres for faces in the pleasant and unpleasant odour conditions, respectively.
- Simultaneous presentation of unpleasant odour and faces boosted effects on hedonic ratings and LPP amplitude relative to independent presentations.
- In unpleasant and no odour conditions, there were differences in the ERP response to faces around the N200 and N400 components. Pleasant odour masked any difference in responses to happy and disgusted faces.

- Faces presented in pleasant or unpleasant odour conditions produced a greater N400 response compared to faces presented without odour. Faces in the unpleasant odour condition produced the greatest N400 amplitude.
- Odour-related activations were observed in the amygdala.
- The superior frontal gyrus and middle frontal gyrus were activated during, and were specifically related to subjective hedonic evaluations of visual stimuli. Activity in the superior frontal gyrus was influenced by pleasant odour.
- Olfactory-visual congruency effects that were also related to subjective hedonic ratings were observed in associative brain regions and the insula.
- Both pleasant and unpleasant odours were rated as more pleasant when paired with happy faces. Pleasant odour paired with pictures of flowers resulted in increased odour pleasantness and intensity ratings and an increase in respiration.
- Unpleasant odour was rated as more intense when it was paired with congruent, disgusted faces. This was accompanied by a decrease in respiration.

7.2 Themes

Several common themes emerged from the experimental findings in the present thesis. The overarching finding was that odour priming effects are robust and bidirectional, using both pleasant and unpleasant odours and different types of visual stimuli. Such effects were represented in behavioural responses, respiratory patterns, ERPs and BOLD signals.

7.2.1 Unpleasant and pleasant odour effects

Taken together, the findings from across experimental chapters suggest a bias of behavioural and neural responses towards unpleasant odour. Unpleasant odour produced stronger amplitude in the N200 and N400 components of the ERP response to faces in comparison to pleasant odour or no odour across two experiments. More

specifically, faces presented simultaneously with unpleasant odour produced stronger amplitude in the LPP in comparison to the same faces presented one-second after unpleasant odour offset. This was accompanied by stronger hedonic ratings. Further, unpleasant odour paired with congruent, disgusted faces resulted in stronger hedonic ratings of face and odour pleasantness, increased odour intensity ratings and a decrease in respiratory amplitude in comparison to when the odour was paired with happy faces. Unpleasant odour was also consistently rated as more intense than pleasant odour during experiments, despite extensive piloting to ensure that the odours were matched on intensity.

These findings correspond with a vast body of literature suggesting that negative, aversive or threatening stimuli elicit more cognitive work, lead to more complex cognitive representations (Peeters & Czapinski, 1990), and evoke strong and rapid physiological, cognitive, emotional, and social responses (Taylor, 1991). The negative valence hypothesis suggests that negative stimuli often evoke stronger responses than positive stimuli of the same intensity (T. A. Ito et al., 1998; Smith et al., 2003; Smith et al., 2006). Indeed, more recent studies have shown greater arousal to losses in comparison to equivalent gains (Sokol-Hessner et al., 2009; Takahashi et al., 2013). In relation to olfactory research, studies have shown that unpleasant odours elicit stronger autonomic arousal than pleasant odours (Brauchli, Ruegg, Etzweiler, & Zeier, 1995), increase the magnitude of the startle reflex (Ehrlichman, Brown Kuhl, Zhu, & Warrenburg, 1997; Ehrlichman, Brown, Zhu, & Warrenburg, 1995; Miltner et al., 1994), and elicit faster behavioural reactions, increasing motor readiness (Bensafi, Rouby, Farget, Vigouroux, & Holley, 2002; Boesveldt et al., 2010; Brauchli et al., 1995). The finding that unpleasant odours consistently resulted in stronger hedonic reactions, ERP responses and changes in respiratory patterns compared to pleasant odour or no odour therefore supports the negative valence hypothesis, and provides further evidence that unpleasant odours evoke stronger reactions than pleasant odours.

Effects of hedonic congruency between unpleasant odour and disgusted faces on subjective ratings and respiratory patterns were observed in Chapter 5. Unpleasant events often prime or amplify responses to other unpleasant stimuli. For example, information about the occurrence of adverse events resulted in an increase in the perceived likelihood of further adverse events (Johnson & Tversky, 1983). Studies

have also shown that negative emotional states increased pessimistic outlooks (Lerner & Keltner, 2001), or perceived likelihood of occurrence of subsequent negative emotional states (DeSteno, Petty, Wegener, & Rucker, 2000). Recent research using odours showed that unpleasant odour increased responses to painful stimuli relative to pleasant odour (Villemure, Slotnick, & Bushnell, 2003). A very recent study showed that unpleasant odour increased aversion and related skin conductance responses to monetary losses (Stancak et al., 2015). These effects were specifically related to odour unpleasantness, as opposed to intensity, and pleasant odour failed to attenuate loss aversion in the same way. Such findings correspond with the effects of affective congruency between prime and target stimuli documented in the evaluative priming literature (Herring et al., 2013; van Reekum et al., 1999). Authors have argued that unpleasant odour likely primes avoidance behaviour, and consequently boosts existing avoidance responses to negative events (Stancak et al., 2015). The results from the present thesis suggest that unpleasant odours primed existing negative responses to disgusted faces, and vice-versa, resulting in changes in face ratings, odour ratings and respiratory patterns.

With regards to pleasant odours, our results suggested that pleasant odours almost always improve ratings of visual stimuli. Neutral faces, happy and disgusted faces, pictures of flowers and objects were all rated as more pleasant when paired with pleasant odours. Happy faces paired with a congruent, pleasant odour were rated as significantly more pleasant than the same faces paired with an unpleasant odour or no odour. However, there was no such interaction when flowers were used as visual stimuli: flowers paired with a congruent, floral odour were not rated significantly more pleasant than the same flowers presented without odour. We interpret this inconsistency as being attributable to the relatedness of the visual stimuli to emotion. Faces are closely linked with emotion, given that they are used to express them (Goldman & Sripada, 2005; Öhman, 2002; Phillips et al., 1997; Walla, 2008). Combined olfactory stimuli and emotional faces may therefore have been more closely related to internal representations of emotion, and as a result, odour-face congruency exerted a greater effect on subjective perception.

The finding that pleasant odours improved ratings of visual stimuli corresponds with a body of behavioural data discussed in the introduction to this thesis (e.g. Baron, 1983, 1990; Dematte et al., 2007; Seubert et al., 2014; Todrank et

al., 1995). However, our EEG data suggested that a pleasant odour masked differences in the ERP response to happy versus disgusted faces. This was a novel finding, and lead to the interpretation that pleasant odours may induce a hedonic state whereby stimuli from other modalities accrue less attentional resources, rendering them less likely to evoke differences in cortical responses. Given the documented effects of pleasant odours on perception and our intuitive experience with pleasant odours, this is perhaps not surprising. Indeed, positively valenced experiences have been shown to attenuate autonomic responses (Ehrlichman et al., 1995; Vrana, Spence, & Lang, 1988). Olfactory research showed that pleasant odours induced positive affect (Baron, 1997), improved mood and perceived health (Knasko, 1992), and did not alter electrocortical activity (Brauchli et al., 1995). A more recent study found that pleasant odour decreased pain (Bartolo et al., 2013). Our finding that pleasant odour overrides differences in cortical responses evoked by information from other modalities is novel, but supported by such research showing that odours induce positive states and reduce autonomic responses.

Data from the fMRI experiment showed a stronger BOLD response to a pleasant, floral odour in comparison to clean air in the amygdala. Such a result provides further evidence for the role of the amygdala in primary olfactory perception (Buck & Bargmann, 2000; Gottfried, 2006). There have been mixed findings in the literature with regards to whether the amygdala encodes odour intensity or odour valence (Anderson et al., 2003; Winston et al., 2005; Zald & Pardo, 1997). Given several of the findings from the present thesis, we argue that the amygdala encodes valence for pleasant odour and visual stimuli: We found greater amygdala activation during hedonic ratings compared to control. Moreover, whilst pleasant odours increased hedonic evaluations of visual stimuli, our data showed amygdala activation in response to pleasant odour, pictures of flowers, and specifically during hedonic ratings of flowers. Hence, our results further support the role of the amygdala in valence encoding (Jin et al., 2015).

Results from the present thesis suggest that whilst pleasant odour reduced differences in cortical responses to visual stimuli, unpleasant odour increased sensitivity to simultaneously presented aversive visual stimuli. Combined stimulation with visual and unpleasant olfactory stimuli resulted in stronger behavioural and cortical responses and changes in respiration, particularly when the olfactory and

visual stimuli were congruent. The results therefore indicate an increased allocation of resources when aversive stimuli from across olfactory and visual modalities are combined, in comparison to when presented alone. We interpreted that such effects likely relate to an evolutionarily adaptive mechanism where cross-modal stimuli interact in the brain to produce a correct behavioural response when aversive stimuli signal danger. Indeed, odours play a role in feeding and mating behaviours (Gottfried, 2006), and serve as warnings about threats in our environment (Paustenbach & Gaffney, 2006; Stevenson, 2010). From an evolutionary perspective, it is adaptive to respond quickly as correctly to adverse events (Taylor, 1991) which may be signalled by odours. Whilst unpleasant odours signal danger, pleasant odours may have the opposite effect. As a result, pleasant and unpleasant odours might respectively increase and decrease sensitivity to congruent and incongruent visual stimuli. Our results suggest that combinations of congruent, ecologically relevant information from across modalities can influence cortical responses, manifesting in behavioural changes represented by subjective ratings and respiratory patterns. Such effects likely reflect evolutionary adaptive mechanisms.

As discussed in Chapter 3, although jasmine odour is consistently rated as pleasant, in its natural form it contains indole, a component perceived as very unpleasant when presented alone (Grabenhorst et al., 2011; Grabenhorst et al., 2007). This counter-intuitive effect appears to happen with many natural odours that are very pleasant (Ohloff, 1994). One study showed that medial orbitofrontal cortex, responsible for representing the pleasantness of odours, responded even more strongly to jasmine when it contained indole compared to when it only contained pleasant components (Grabenhorst et al., 2007). This led to the suggestion that brain areas representing the pleasantness of stimuli can do so in a way that is partly independent of unpleasant components, thereby emphasising the pleasant component of a hedonically complex mixture. However, a later study showed that activity in the superior frontal gyrus increased when selective attention was being paid to jasmine without indole, and also when no selective attention was required but the jasmine did contain indole (Grabenhorst et al., 2011). The authors proposed the new hypothesis that the affective potency of stimuli with mixed pleasant and unpleasant components is related to the recruitment of mechanisms in the brain involved in attentional capture. The effects of the jasmine odour observed in the present experiments might

be related to hedonic complexity or attentional capture; however, this seems unlikely given that the concentration of indole in the presently used jasmine odour was only 0.024%.

7.2.2 Bidirectional cross-modal effects of odours and visual stimuli

Another interesting theme emerging from the present studies is the observation of bidirectional cross-modal effects of olfactory and visual stimuli: Odours affected ratings of visual stimuli, and in turn visual stimuli exerted cross-modal effects on odour pleasantness and intensity perception. A meta-analysis including evaluative conditioning studies using a range of cross-modal stimuli, including pictures, sounds, odours and tastes, as conditioned and unconditioned stimuli was conducted by Hofmann et al. (2010). They showed that evaluative conditioning was independent of whether or not stimuli were matched on modality, and argued for the generality of representations of cross-modal contingencies (Hofmann et al., 2010). Our research supports and extends these findings by showing that odours and visual stimuli were effective as both primes and targets, in priming responses to both odours and visual stimuli.

In the experiments discussed, happy and disgusted faces and pictures of flowers influenced ratings of odour pleasantness and intensity, replicating previous findings (Pollatos et al., 2007; Seo, Arshamian, et al., 2010) and extending them to the use of emotional faces as primes. Again, congruency amplified these effects, in particular for combined unpleasant odour and disgusted faces. This highlights the importance of congruency in olfactory-visual interactions, and provides further support for the negative valence hypothesis. Moreover, pictures of flowers served as a pleasant, odour-congruent visual stimulus, and resulted in increased pleasantness and intensity ratings for both pleasant odour and clean air, and effects were amplified when the task focus was related to hedonic value, further suggesting that affect-related cognitive demand is important in perception (Rolls et al., 2008).

Relating to bidirectional cross-modal effects of olfactory and visual stimuli, the experiments discussed observed differences in respiratory patterns related to olfactory-visual integration, and olfactory-visual congruency. Results showed a

decrease in the amplitude of respiratory movements when unpleasant odour was paired with simultaneously presented disgusted faces, and an increase in respiratory amplitude during pleasant odour trials and trials showing pictures of flowers. Odours are tightly linked with emotions (Ehrlichman & Halpern, 1988), and emotions are closely associated with approach or avoidance behaviours (Barrett, 2006, 2009; Frijda, 1988). In general, our results suggest that participants increased their respiratory movements in response to pleasant odour and odour-related visual stimuli, representative of approach behaviour. Participants decreased respiratory movements in response to negative odours, in particular when combined with negative visual stimuli, representing avoidance behaviour. These results therefore further support the evolutionary argument for the role of odours as warnings about potential dangers in the environment and elicitors of adaptive approach/avoidance responses (Gottfried, 2006; Paustenbach & Gaffney, 2006; Stevenson, 2010; Taylor, 1991). We contribute that approach/avoidance behaviours manifest in changes in respiratory patterns in response to ecologically relevant cross-modal information.

7.2.3 Implications for evaluative priming and evaluative conditioning

As mentioned above, the results from the present thesis contribute to a more general literature on evaluative priming and evaluative conditioning, which respectively refer to automatic and conditioned changes in affective responses to stimuli when paired with other positively and negatively valenced stimuli (Dirk Hermans & Baeyens, 2002; Herring et al., 2013; Hofmann et al., 2010). Our findings support and update early research suggesting that evaluative priming effects exist in a cross-modal sense, where pleasant and unpleasant odours influence immediate responses to other stimuli (Dirk Hermans & Baeyens, 2002). Our data further suggest that olfactory-visual congruency is important in these effects, and expand the finding that evaluative priming effects (particularly congruency) are represented in the N400 and later ERP components (Aguado et al., 2013; Herring et al., 2011) by further showing that this is also true for olfactory-visual evaluative priming (Zhang et al., 2010). Moreover, a recent meta-analysis argued that both encoding and response mechanisms are at play during evaluative priming (Herring et al., 2013). Encoding

mechanisms activate object-evaluation associations in memory that make the valence of targets more accessible, whilst response mechanisms influence the ease with which a person can generate a response to the target. The fact that results from the present thesis observed effects of evaluative congruency in ERPs and BOLD responses, likely encoding, as well as in subjective responses, support and extend this argument to cross-modal evaluative priming. Further, the bidirectionality of olfactory-visual effects observed in the present experiments lends support to holistic accounts of evaluative conditioning, which suggest that co-occurrences of conditioned and unconditioned stimuli activate holistic representations that result in associative stimulus evaluations (Hofmann et al., 2010; Levey & Martin, 1975; Martin & Levey, 1994).

7.2.4 Implications for decision making

The present thesis further relates to the literature on decision making. The experiments discussed analysed changes in pleasantness ratings that occurred as a result of odour stimulation. Pleasantness ratings cannot be considered as decisions per se, as these involve commitment to a binary choice. However, pleasantness ratings reveal subjective valuations, which can provide a basis for decision making (Kühn & Gallinat, 2012; Lebreton et al., 2009). Values expressed by subjective ratings are unreliable, vary over short periods of time and can be easily manipulated (Abitbol et al., 2015; Bardsley, 2010; Kahneman & Tversky, 2000; McFadden, 2005). Affective context can influence subjective valuations, and therefore, decision making. In the experiments discussed, odours therefore represent an indirect induction of affective context that results as an incidental factor in subjective valuations, and potentially decision making (Loewenstein & Lerner, 2003; Raghunathan & Pham, 1999). Our results showed that odours and olfactory-visual stimulus pairs have a robust effect on subjective valuations, affecting subjective hedonic ratings of faces, flowers and objects, ratings of facial expression, and decisions regarding odour pleasantness and intensity perception. As discussed, olfactory-visual congruency is important in these effects, and tends to increase their magnitude. Such findings therefore support the role of olfactory-visual congruency in decision making (Mitchell, Kahn, & Knasko, 1995).

Importantly, we observed BOLD responses in the dmPFC, a region typically referred to as part of the brain's valuation system, consistently activated during decision making about subjective value (Bartra et al., 2013; Lebreton et al., 2013; Rushworth, Kolling, Sallet, & Mars, 2012). Our results support and extend findings from Abitbol et al. (2015), who suggested that fluctuations in pre-stimulus activity induced by an external context (pleasant music) could serve as a source of variability in subjective valuation and related activity in the brain's valuation system. We showed that responses in the dmPFC were specific to a hedonic rating condition in comparison to control, were directly related to subjective ratings, and could be boosted by a pleasant odour context. The present thesis therefore contributes that olfactory-visual interactions are relevant in decision making, where activity in the brain valuation system during decision making can be modulated by a pleasant odour context.

Prospect theory proposes that losses acquire more weight than equivalent gains during decision making (Tversky & Kahneman, 1992). Loss aversion is defined as the tendency to prefer avoiding losses over acquiring equivalent gains (Tversky & Kahneman, 1991). Previous research investigated the effects of odours on loss aversion, and suggested that unpleasant odours increased such effects (Stancak et al., 2015). As discussed above, the present experiments observed a bias towards unpleasant odour and unpleasant olfactory-visual stimulus pairs, manifesting in modulations of subjective value and respiratory patterns. Although not directly comparable with losses, both unpleasant odour and disgusted faces may signal adverse events (Stevenson, 2010; Walla, 2008) that could result in losses. The notable bias towards unpleasant odour and olfactory-visual stimulus pairs in the present experiments may therefore relate to a greater attention towards losses as suggested by prospect and loss aversion theories.

Moreover, the present findings suggest that a general focus on hedonics has a role in decision making about odour perception. Results showed that pleasant odour was rated as more pleasant and more intense when the experimental trial required a hedonic rating of a visual stimulus, further suggesting that affective focus is important in cognition and decision making (Rolls et al., 2008).

7.3 Further theoretical and practical implications

As indicated by the themes discussed above, the findings of the present thesis have potential applications across multiple disciplines. The findings have implications for theories of basic neuroscience; with regards to how the transfer of affective information from across modalities is represented in the brain, and how this relates to behaviour. From an evolutionary perspective, the results suggest that cross-modal stimuli combine to produce evolutionarily adaptive neural responses, and influence subsequent behaviour. From a clinical perspective, progress in the clarification of central olfactory processing relating to decision making in the healthy human brain may inform understanding of neurological disease. Neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease induce olfactory impairments in the early course of illness, which often precede the onset of other symptoms (Gottfried, 2006; Hawkes, 2003; Mesholam, Moberg, Mahr, & Doty, 1998; Murphy, 1999). Increased knowledge about the functional organisation of olfaction may eventually lead to diagnostic and treatment interventions for such illnesses.

This research may also have implications in a commercial setting where odours are used as part of fragranced products for home care, personal care and laundry. The results highlight the importance of odour valence, congruency between odours and visual stimuli, and bidirectional effects of odours and visual stimuli. Thus, such mechanisms could be taken into consideration during the development of product packaging and marketing. In terms of methodological implications, the use of SPM for analysis of ERPs in the present thesis has proved a robust and sensitive means to analysing data in an exploratory fashion, and should be considered in future EEG research.

7.4 Limitations

The EEG experiments in the current thesis were limited to the investigation of the ERP response to faces. It may have also been useful to investigate olfactory ERPs, or pre-stimulus oscillatory activity that occurred before face presentation that

may have been related to odour priming. Such analyses could have helped to further dissociate genuine odour priming from potential misattributed responses to odours themselves. However, ERPs are often used to investigate emotional components of the response to faces (Bentin et al., 1996; Cacioppo et al., 1993; Duval et al., 2013), and are used as a standard measure in the evaluative priming literature (Bensafi, Pierson, et al., 2002; Herrmann et al., 2000; Hietanen & Astikainen, 2013; Zhang, Lawson, Guo, & Jiang, 2006). The focus of the current project was to investigate the ERP response to faces under odour conditions, and to further validate the use of SPM as a novel and exploratory approach to the investigation. Hence, this ERP analysis method was substantial for a three year research project, and further analysis would not have been viable within the time frame.

As with all imaging research, the present thesis was limited by the inherent spatial limitations of neuroimaging. Regions of neural activation are approximate rather than exact, and this must be considered during interpretation of the findings. As highlighted in Chapter 6, imaging of the OFC is affected by susceptibility gradients in echo-planer imaging (Deichmann et al., 2003), which may have resulted in signal loss in the fMRI investigation in the present thesis. Thus, alternative scanning parameters may have been preferable.

An obvious limitation common across all experimental chapters, and to most studies of this nature, is that the participants were predominantly undergraduate and postgraduate students. Small samples from this group may not be the most representative for generalising results to the wider population (Henrich, Heine, & Norenzayan, 2010), so results should be treated with caution until they have been replicated cross-culturally. Further, the effects observed in the present research may differ as a function of the gender and age of the participants. Such differences were not explored in the present thesis due to time constraints, but would make an interesting subject for further study.

7.5 Suggestions for future research

In addition to exploring gender and age differences, it may also be interesting to investigate odour priming using different odour intensities or odour mixtures in

future research. Seubert et al. (2014) found that several odour mixtures, linearly increasing from unpleasant to pleasant, induced a corresponding linear increase in face attractiveness ratings. However, no EEG data was collected. Future research could seek to investigate the neural mechanisms underlying such effects of odour mixtures, with particular emphasis on hedonic thresholds. It could be interesting to observe the thresholds at which odour mixtures are perceived as pleasant or unpleasant, when they begin to affect ratings of faces according to perceived pleasantness, and how neural activity correlates with such effects. Moreover, future research could address whether different emotional faces (e.g. happy and disgusted) affect the threshold at which an odour mixture is perceived as pleasant or unpleasant, or increase/decrease the threshold at which odours are consciously perceived. Investigating the neural mechanisms underlying these effects, and exploring how they relate to odour priming would make a valuable contribution to the present research and existing literature.

The present findings are limited by the small selection of odours used in the experiments, owing to the complexity and length of odour experiments, which make it difficult to administer many different odours in one task. Future research should aim to employ a greater range of different odours. Furthermore, future research could address the role of habituation in odour priming. In Chapter 3, we observed a gradual decrease in hedonic effects of odours across experimental blocks, particularly with the unpleasant odour. Future studies could introduce time as an experimental variable and employ single-trial analysis of ERPs to investigate the role of habituation in odour priming.

There is a vast scope for further fMRI investigations relating to the findings in the present thesis. Such investigations could employ dynamic causal modelling (DCM) analysis (Friston, Harrison, & Penny, 2003) or psychophysiological interaction (PPI) analysis (Friston et al., 1997) to observe the interplay between primary and secondary olfactory areas and the brain valuation system (e.g. the amygdala, OFC and dmPFC) in olfactory-visual interactions, and how these interactions relate to subjective ratings in hedonic versus perceptual rating tasks. Recent studies have pointed to the involvement of both OFC and amygdala in the representation of subjective valence for stimuli across modalities (Chikazoe, Lee, Kriegeskorte, & Anderson, 2014; Jin et al., 2015). However, the findings are

somewhat mixed with regards to whether activity in the OFC and amygdala support a unique functionality (Jin et al., 2015). Future investigations of olfactory-visual interactions using PPI and/or DCM could be used to address this question.

7.6 Concluding remarks

Odours and emotion are closely linked due to the overlaps between olfactory and emotional systems in the brain. The present thesis employed novel paradigms and analysis methods to investigate the neural mechanisms underlying the effects of odours on hedonic and emotional perception. It is clear that odours influence evaluations of visual stimuli. Visual stimuli presented with unpleasant odours evoke strong neural and behavioural responses manifesting in changes in subjective ratings and respiratory patterns, which are likely evolutionarily adaptive. Further, in the same way that odours influence evaluations of visual stimuli, visual stimuli influence odour pleasantness and intensity perception. Olfactory-visual interactions are often dependent on congruency, and are represented in late ERP components and associative brain regions located in the frontal cortex. Results from the present thesis provide further support for the role of the frontal cortex, namely dmPFC in subjective evaluations, and suggest that related activations are mediated by odour context. The present thesis expands previous findings and offers new insights to cross-modal, evaluative priming, decision making and reward processing literatures. It is hoped that the findings provide a basis for future neuroimaging research addressing the cross-modal effects of odours, and the corresponding representation of valence in the brain.

References

Abitbol, R., Lebreton, M., Hollard, G., Richmond, B. J., Bouret, S., & Pessiglione, M. (2015). Neural Mechanisms Underlying Contextual Dependency of Subjective Values: Converging Evidence from Monkeys and Humans. *The Journal of Neuroscience, 35*(5), 2308-2320. doi:10.1523/JNEUROSCI.1878-14.2015

Adolph, D., Meister, L., & Pause, B. M. (2013). Context counts! social anxiety modulates the processing of fearful faces in the context of chemosensory anxiety signals. *Frontiers in Human Neuroscience, 7*. doi:Artn 283

Doi 10.3389/Fnhum.2013.00283

Adrian, E. (1950). The electrical activity of the mammalian olfactory bulb. *Electroencephalography and clinical neurophysiology, 2*(1-4), 377-388.

Aguado, L., Dieguez-Risco, T., Mendez-Bertolo, C., Pozo, M. A., & Hinojosa, J. A. (2013). Priming effects on the N400 in the affective priming paradigm with facial expressions of emotion. *Cogn Affect Behav Neurosci, 13*(2), 284-296. doi:10.3758/s13415-012-0137-3

Ahern, G. L., & Schwartz, G. E. (1985). Differential lateralization for positive and negative emotion in the human brain: EEG spectral analysis. *Neuropsychologia, 23*(6), 745-755. doi:10.1016/0028-3932(85)90081-8

Aminoff, E. M., Kveraga, K., & Bar, M. (2013). The role of the parahippocampal cortex in cognition. *Trends in cognitive sciences, 17*(8), 379-390. doi:10.1016/j.tics.2013.06.009

Anderson, A. K., Christoff, K., Stappen, I., Panitz, D., Ghahremani, D. G., Glover, G., . . . Sobel, N. (2003). Dissociated neural representations of intensity and valence in human olfaction. *Nat Neurosci, 6*(2), 196-202. doi:10.1038/nn1001

Attwell, D., & Iadecola, C. (2002). The neural basis of functional brain imaging signals. *Trends Neurosci, 25*(12), 621-625. doi:http://dx.doi.org/10.1016/S0166-2236(02)02264-6

Aurlien, H., Gjerde, I. O., Aarseth, J. H., Eldoen, G., Karlsen, B., Skeidsvoll, H., & Gilhus, N. E. (2004). EEG background activity described by a large computerized database. *Clin Neurophysiol, 115*(3), 665-673. doi:10.1016/j.clinph.2003.10.019

Ball, L. J., Shoker, J., & Miles, J. N. V. (2010). Odour-based context reinstatement effects with indirect measures of memory: The curious case of rosemary. *British Journal of Psychology, 101*(4), 655-678.

Ball, T., Derix, J., Wentlandt, J., Wieckhorst, B., Speck, O., Schulze-Bonhage, A., & Mutschler, I. (2009). Anatomical specificity of functional amygdala imaging of responses to stimuli with positive and negative emotional valence. *J Neurosci Methods, 180*(1), 57-70. doi:10.1016/j.jneumeth.2009.02.022

Banks, S. J., Ng, V., & Jones-Gotman, M. (2012). Does good+good=better? A pilot study on the effect of combining hedonically valenced smells and images. *Neurosci Lett, 514*(1), 71-76. doi:10.1016/j.neulet.2012.02.059

Bardsley, N. (2010). *Experimental economics: Rethinking the rules*: Princeton University Press.

Baron, R. A. (1983). " Sweet smell of success"? The impact of pleasant artificial scents on evaluations of job applicants. *Journal of Applied Psychology, 68*(4), 709. doi:10.1037/0021-9010.68.4.709

Baron, R. A. (1990). Environmentally Induced Positive Affect: Its Impact on Self-Efficacy, Task Performance, Negotiation, and Conflict1. *Journal of Applied Social Psychology, 20*(5), 368-384.

Baron, R. A. (1997). The sweet smell of... helping: Effects of pleasant ambient fragrance on prosocial behavior in shopping malls. *Personality and Social Psychology Bulletin, 23*, 498-503.

Barrett, L. F. (2006). Solving the emotion paradox: Categorization and the experience of emotion. *Personality and social psychology review, 10*(1), 20-46.

Barrett, L. F. (2009). Variety is the spice of life: A psychological construction approach to understanding variability in emotion. *Cognition and Emotion, 23*(7), 1284-1306.

Bartolo, M., Serrao, M., Gamgebeli, Z., Alpaidze, M., Perrotta, A., Padua, L., . . . Sandrini, G. (2013). Modulation of the human nociceptive flexion reflex by pleasant and unpleasant odors. *PAIN®, 154*(10), 2054-2059.

Bartra, O., McGuire, J. T., & Kable, J. W. (2013). The valuation system: a coordinate-based meta-analysis of BOLD fMRI experiments examining neural correlates of subjective value. *Neuroimage, 76*, 412-427.

Batty, M., & Taylor, M. J. (2003). Early processing of the six basic facial emotional expressions. *Cognitive Brain Research, 17*(3), 613-620. doi:http://dx.doi.org/10.1016/S0926-6410(03)00174-5

Bensafi, M., Pierson, A., Rouby, C., Farget, V., Bertrand, B., Vigouroux, M., . . . Holley, A. (2002). Modulation of visual event-related potentials by emotional olfactory stimuli. *Neurophysiologie Clinique-Clinical Neurophysiology, 32*(6), 335-342. doi:Pii S0987-7053(02)00337-4

Doi 10.1016/S0987-7053(02)00337-4

Bensafi, M., Rouby, C., Farget, V., Bertrand, B., Vigouroux, M., & Holley, A. (2002). Influence of affective and cognitive judgments on autonomic parameters during inhalation of pleasant and unpleasant odors in humans. *Neurosci Lett, 319*(3), 162-166. doi:10.1016/S0304-3940(01)02572-1

Bensafi, M., Rouby, C., Farget, V., Vigouroux, M., & Holley, A. (2002). Asymmetry of pleasant vs. unpleasant odor processing during affective judgment in humans. *Neurosci Lett, 328*(3), 309-313.

Bentin, S., Allison, T., Puce, A., Perez, E., & McCarthy, G. (1996). Electrophysiological studies of face perception in humans. *Journal of cognitive neuroscience, 8*(6), 551-565. doi:10.1162/jocn.1996.8.6.551

Bentin, S., & Deouell, L. Y. (2000). Structural encoding and identification in face processing: ERP evidence for separate mechanisms. *Cognitive Neuropsychology, 17*(1-3), 35-55.

Berg, P., & Scherg, M. (1994). A multiple source approach to the correction of eye artifacts. *Electroencephalography and clinical neurophysiology, 90*(3), 229-241. doi:http://dx.doi.org/10.1016/0013-4694(94)90094-9

Boeijinga, P., & Da Silva, F. L. (1988). Differential distribution of β and θ EEG activity in the entorhinal cortex of the cat. *Brain research, 448*(2), 272-286.

Boeijinga, P., & da Silva, F. L. (1989). Modulations of EEG activity in the entorhinal cortex and forebrain olfactory areas during odour sampling. *Brain research, 478*(2), 257-268.

Boesveldt, S., Frasnelli, J., Gordon, A. R., & Lundstrom, J. N. (2010). The fish is bad: Negative food odors elicit faster and more accurate reactions than other odors. *Biol Psychol, 84*(2), 313-317. doi:10.1016/j.biopsycho.2010.03.006

Boiten, F. A., Frijda, N. H., & Wientjes, C. J. E. (1994). Emotions and respiratory patterns: review and critical analysis. *International Journal of Psychophysiology, 17*(2), 103-128. doi:http://dx.doi.org/10.1016/0167-8760(94)90027-2

Bone, P. F., & Jantrania, S. (1992). Olfaction as a cue for product quality. *Marketing Letters, 3*(3), 289-296.

Brauchli, P., Ruegg, P. B., Etzweiler, F., & Zeier, H. (1995). Electrocortical and autonomic alteration by administration of a pleasant and an unpleasant odor. *Chem Senses, 20*(5), 505-515.

Bressler, S. L. (1987a). Relation of olfactory bulb and cortex. I. Spatial variation of bulbocortical interdependence. *Brain research, 409*(2), 285-293.

Bressler, S. L. (1987b). Relation of olfactory bulb and cortex. II. Model for driving of cortex by bulb. *Brain research, 409*(2), 294-301.

Bressler, S. L., & Freeman, W. J. (1980). Frequency analysis of olfactory system EEG in cat, rabbit, and rat. *Electroencephalography and clinical neurophysiology, 50*(1), 19-24.

Brett, M., Anton, J.-L., Valabregue, R., & Poline, J.-P. (2002). Region of interest analysis using an SPM toolbox [abstract]. *8th International Conference on Functional Mapping of the Human Brain,*.

Buchanan, T. W., Tranel, D., & Adolphs, R. (2003). A specific role for the human amygdala in olfactory memory. *Learning & Memory, 10*(5), 319-325.

Buck, L. B., & Bargmann, C. (2000). Smell and taste: the chemical senses. *Principles of neural science, 4*, 625-647.

Cacioppo, J. T., Crites, S. L., Berntson, G. G., & Coles, M. G. H. (1993). If attitudes affect how stimuli are processed, should they not affect the event-related brain potential? *Psychological Science, 4*(2), 108-112.

Canli, T., Desmond, J. E., Zhao, Z., Glover, G., & Gabrieli, J. D. E. (1998). Hemispheric asymmetry for emotional stimuli detected with fMRI. *Neuroreport, 9*(14), 3233-3239. doi:10.1097/00001756-199810050-00019

Carmichael, S. T., Clugnet, M. C., & Price, J. L. (1994). Central olfactory connections in the macaque monkey. *J Comp Neurol, 346*(3), 403-434. doi:10.1002/cne.903460306

Castle, P. C., Van Toller, S., & Milligan, G. J. (2000). The effect of odour priming on cortical EEG and visual ERP responses. *Int J Psychophysiol, 36*(2), 123-131.

Cauquil, A. S., Edmonds, G. E., & Taylor, M. J. (2000). Is the face-sensitive N170 the only ERP not affected by selective attention? *Neuroreport, 11*(10), 2167-2171.

Chikazoe, J., Lee, D. H., Kriegeskorte, N., & Anderson, A. K. (2014). Population coding of affect across stimuli, modalities and individuals. *Nat Neurosci, 17*(8), 1114-1122. doi:10.1038/nn.3749

Cloutman, L. L., Binney, R. J., Drakesmith, M., Parker, G. J. M., & Lambon Ralph, M. A. (2012). The variation of function across the human insula mirrors its patterns of structural connectivity: Evidence from in vivo probabilistic tractography. *Neuroimage, 59*(4), 3514-3521. doi:http://dx.doi.org/10.1016/j.neuroimage.2011.11.016

Cook, S., Fallon, N., Wright, H., Thomas, A., Giesbrecht, T., Field, M., & Stancak, A. (2015). Pleasant and Unpleasant Odors Influence Hedonic Evaluations of Human Faces: An Event-Related Potential Study. *Front. Hum. Neurosci., 9*, 661. doi:10.3389/fnhum.2015.00661

Cook, S., Kokmotou, K., Soto, V., Fallon, N., Thomas, A., Giesbrecht, T., . . . Stancak, A. (in preparation). Pleasant and unpleasant odour-face combinations influence face and odour perception: an event-related potential study. *Biol Psychol*.

Cook, S., Kokmotou, K., Soto, V., Wright, H., Fallon, N., Thomas, A., . . . Stancak, A. (under review). Effects of stimulus onset asynchrony on odour induced hedonic evaluations of faces: an event-related potential study. *European Journal of Neuroscience*.

Cowley, J. J., Johnson, A. L., & Brooksbank, B. W. L. (1977). The effect of two odorous compounds on performance in an assessment-of-people test. *Psychoneuroendocrinology, 2*(2), 159-172.

Critchley, H. D., & Rolls, E. T. (1996). Olfactory neuronal responses in the primate orbitofrontal cortex: analysis in an olfactory discrimination task. *Journal of Neurophysiology, 75*(4), 1659-1672.

Croy, I., Drechsler, E., Hamilton, P., Hummel, T., & Olausson, H. (2016). Olfactory modulation of affective touch processing — A neurophysiological

investigation. *Neuroimage, 135*, 135-141. doi:http://dx.doi.org/10.1016/j.neuroimage.2016.04.046

Cuthbert, B. N., Schupp, H. T., Bradley, M. M., Birbaumer, N., & Lang, P. J. (2000). Brain potentials in affective picture processing: covariation with autonomic arousal and affective report. *Biol Psychol, 52*(2), 95-111. doi:10.1016/S0301-0511(99)00044-7

Dade, L. A., Zatorre, R. J., & Jones-Gotman, M. (2002). Olfactory learning: convergent findings from lesion and brain imaging studies in humans. *Brain, 125*(1), 86-101.

Dalton, P., Mauté, C., Jaén, C., & Wilson, T. (2013). Chemosignals of stress influence social judgments. *PLoS One, 8*(10), e77144.

Davidson, R. J. (1998). Affective style and affective disorders: Perspectives from affective neuroscience. *Cognition Emotion, 12*(3), 307-330. doi:10.1080/026999398379628

de Araujo, I. E., Rolls, E. T., Velazco, M. I., Margot, C., & Cayeux, I. (2005). Cognitive modulation of olfactory processing. *Neuron, 46*(4), 671-679.

De Houwer, D. H., Paul. Eelen, Jan. (1998). Affective and Identity Priming with Episodically Associated Stimuli. *Cogn. Emot., 12*(2), 145-169. doi:10.1080/026999398379691

Deichmann, R., Gottfried, J. A., Hutton, C., & Turner, R. (2003). Optimized EPI for fMRI studies of the orbitofrontal cortex. *Neuroimage, 19*(2), 430-441. doi:http://dx.doi.org/10.1016/S1053-8119(03)00073-9

Dematte, M. L., Osterbauer, R., & Spence, C. (2007). Olfactory cues modulate facial attractiveness. *Chem Senses, 32*(6), 603-610. doi:10.1093/chemse/bjm030

Dematte, M. L., Sanabria, D., & Spence, C. (2009). Olfactory discrimination: when vision matters? *Chem Senses, 34*(2), 103-109. doi:10.1093/chemse/bjn055

DeSteno, D., Petty, R. E., Wegener, D. T., & Rucker, D. D. (2000). Beyond valence in the perception of likelihood: the role of emotion specificity. *J Pers Soc Psychol, 78*(3), 397.

Dieguez-Risco, T., Aguado, L., Albert, J., & Hinojosa, J. A. (2013). Faces in context: modulation of expression processing by situational information. *Soc Neurosci, 8*(6), 601-620. doi:10.1080/17470919.2013.834842

Doty, R. L. (1975). An examination of relationships between the pleasantness, intensity, and concentration of 10 odorous stimuli. *Perception & Psychophysics, 17*(5), 492-496. doi:10.3758/bf03203300

Doty, R. L., Shaman, P., Kimmelman, C. P., & Dann, M. S. (1984). University of Pennsylvania Smell Identification Test: a rapid quantitative olfactory function test for the clinic. *Laryngoscope, 94*(2 Pt 1), 176-178.

Durand, K., Baudouin, J. Y., Lewkowicz, D. J., Goubet, N., & Schaal, B. (2013). Eye-catching odors: olfaction elicits sustained gazing to faces and eyes in 4-month-old infants. *PLoS One, 8*(8), e70677. doi:10.1371/journal.pone.0070677

Duval, E. R., Moser, J. S., Huppert, J. D., & Simons, R. F. (2013). What's in a face? The late positive potential reflects the level of facial affect expression. *J. Psychophysiol., 27*(1), 27. doi:10.1027

Ehrlichman, H., Brown Kuhl, S., Zhu, J., & Warrenburg, S. (1997). Startle reflex modulation by pleasant and unpleasant odors in a between-subjects design. *Psychophysiology, 34*(6), 726-729.

Ehrlichman, H., Brown, S., Zhu, J., & Warrenburg, S. (1995). Startle reflex modulation during exposure to pleasant and unpleasant odors. *Psychophysiology, 32*(2), 150-154.

Ehrlichman, H., & Halpern, J. N. (1988). Affect and memory: effects of pleasant and unpleasant odors on retrieval of happy and unhappy memories. *J Pers Soc Psychol, 55*(5), 769-779.

Eimer, M. (2000). The face-specific N170 component reflects late stages in the structural encoding of faces. *Neuroreport, 11*(10), 2319-2324.

Eimer, M., & Holmes, A. (2007). Event-related brain potential correlates of emotional face processing. *Neuropsychologia, 45*(1), 15-31. doi:http://dx.doi.org/10.1016/j.neuropsychologia.2006.04.022

Eimer, M., Holmes, A., & McGlone, F. P. (2003). The role of spatial attention in the processing of facial expression: an ERP study of rapid brain responses to six basic emotions. *Cogn Affect Behav Neurosci, 3*(2), 97-110.

Ekstrom, A. (2010). How and when the fMRI BOLD signal relates to underlying neural activity: the danger in dissociation. *Brain Res Rev, 62*(2), 233-244. doi:10.1016/j.brainresrev.2009.12.004

Elliott, R., Dolan, R. J., & Frith, C. D. (2000). Dissociable Functions in the Medial and Lateral Orbitofrontal Cortex: Evidence from Human Neuroimaging Studies. *Cerebral cortex, 10*(3), 308-317. doi:10.1093/cercor/10.3.308

Engen, T. (1973). The sense of smell. *Annu Rev Psychol, 24*, 187-206. doi:10.1146/annurev.ps.24.020173.001155

Engen, T., & Ross, B. M. (1973). Long-term memory of odors with and without verbal descriptions. *J Exp Psychol, 100*(2), 221-227.

Fallon, N. J., J.; Giesbrecht, T.; Thomas, A.; Stancak, A. (in preparation). Olfactory habituation during congruent visual stimulation.

Firestein, S. (2001). How the olfactory system makes sense of scents. *Nature, 413*(6852), 211-218. doi:10.1038/35093026

Fisch, B. J. (1999). *Fisch and Spehlmann's EEG primer : basic principles of digital and analog EEG* (3rd rev. and enlarged ed.). Amsterdam ; Oxford: Elsevier.

Folstein, J. R., & Van Petten, C. (2008). Influence of cognitive control and mismatch on the N2 component of the ERP: a review. *Psychophysiology, 45*(1), 152-170. doi:10.1111/j.1469-8986.2007.00602.x

Fox, P. T., & Raichle, M. E. (1986). Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proc Natl Acad Sci U S A, 83*(4), 1140-1144.

Frijda, N. H. (1988). The laws of emotion. *American psychologist, 43*(5), 349.

Friston, K. J. (2004). Experimental design and statistical parametric mapping.

Friston, K. J., Buechel, C., Fink, G., Morris, J., Rolls, E., & Dolan, R. (1997). Psychophysiological and modulatory interactions in neuroimaging. *Neuroimage, 6*(3), 218-229.

Friston, K. J., Harrison, L., & Penny, W. (2003). Dynamic causal modelling. *Neuroimage, 19*(4), 1273-1302.

Friston, K. J., Holmes, A. P., Worsley, K. J., Poline, J. P., Frith, C. D., & Frackowiak, R. S.-J. (1994). Statistical parametric maps in functional imaging: a general linear approach. *Human brain mapping, 2*(4), 189-210.

Gauthier, I., Curran, T., Curby, K. M., & Collins, D. (2003). Perceptual interference supports a non-modular account of face processing. *Nat Neurosci, 6*(4), 428-432.

Goldman, A. I., & Sripada, C. S. (2005). Simulationist models of face-based emotion recognition. *Cognition, 94*(3), 193-213. doi:http://dx.doi.org/10.1016/j.cognition.2004.01.005

Gomez, P., Stahel, W. A., & Danuser, B. (2004). Respiratory responses during affective picture viewing. *Biol Psychol, 67*(3), 359-373. doi:http://dx.doi.org/10.1016/j.biopsycho.2004.03.013

Gomez, P., Zimmermann, P., Guttormsen-Schär, S., & Danuser, B. (2005). Respiratory responses associated with affective processing of film stimuli. *Biol Psychol, 68*(3), 223-235. doi:http://dx.doi.org/10.1016/j.biopsycho.2004.06.003

Gottfried, J. A. (2006). Smell: central nervous processing. *Adv Otorhinolaryngol, 63*, 44-69. doi:10.1159/000093750

Gottfried, J. A., Deichmann, R., Winston, J. S., & Dolan, R. J. (2002). Functional heterogeneity in human olfactory cortex: an event-related functional magnetic resonance imaging study. *The Journal of Neuroscience, 22*(24), 10819-10828.

Gottfried, J. A., & Dolan, R. J. (2003). The nose smells what the eye sees: crossmodal visual facilitation of human olfactory perception. *Neuron, 39*(2), 375-386.

Gottfried, J. A., & Dolan, R. J. (2004). Human orbitofrontal cortex mediates extinction learning while accessing conditioned representations of value. *Nat Neurosci, 7*(10), 1144-1152.

Gottfried, J. A., O'Doherty, J., & Dolan, R. J. (2002). Appetitive and aversive olfactory learning in humans studied using event-related functional magnetic resonance imaging. *The Journal of Neuroscience, 22*(24), 10829-10837.

Gottfried, J. A., O'Doherty, J., & Dolan, R. J. (2003). Encoding predictive reward value in human amygdala and orbitofrontal cortex. *Science, 301*(5636), 1104-1107.

Gottfried, J. A., Smith, A. P., Rugg, M. D., & Dolan, R. J. (2004). Remembrance of odors past: human olfactory cortex in cross-modal recognition memory. *Neuron, 42*(4), 687-695.

Gottfried, J. A., & Zald, D. H. (2005). On the scent of human olfactory orbitofrontal cortex: Meta-analysis and comparison to non-human primates. *Brain*

Research Reviews, 50(2), 287-304. doi:http://dx.doi.org/10.1016/j.brainresrev.2005.08.004

Grabenhorst, F., D'Souza, A. A., Parris, B. A., Rolls, E. T., & Passingham, R. E. (2010). A common neural scale for the subjective pleasantness of different primary rewards. *Neuroimage, 51*(3), 1265-1274. doi:10.1016/j.neuroimage.2010.03.043

Grabenhorst, F., & Rolls, E. T. (2009). Different representations of relative and absolute subjective value in the human brain. *Neuroimage, 48*(1), 258-268. doi:http://dx.doi.org/10.1016/j.neuroimage.2009.06.045

Grabenhorst, F., & Rolls, E. T. (2011). Value, pleasure and choice in the ventral prefrontal cortex. *Trends in cognitive sciences, 15*(2), 56-67.

Grabenhorst, F., Rolls, E. T., & Margot, C. (2011). A hedonically complex odor mixture produces an attentional capture effect in the brain. *Neuroimage, 55*(2), 832-843. doi:http://dx.doi.org/10.1016/j.neuroimage.2010.12.023

Grabenhorst, F., Rolls, E. T., Margot, C., da Silva, M. A., & Velazco, M. I. (2007). How pleasant and unpleasant stimuli combine in different brain regions: odor mixtures. *J Neurosci, 27*(49), 13532-13540. doi:10.1523/jneurosci.3337-07.2007

Grabenhorst, F., Rolls, E. T., & Parris, B. A. (2008). From affective value to decision-making in the prefrontal cortex. *European Journal of Neuroscience, 28*(9), 1930-1939.

Grigor, J. A. (1995). Do the Eyes See What the Nose Knows - an Investigation of the Effects of Olfactory Priming on Visual Event-Related Potentials. *Chem Senses, 20*(1), 163-163.

Grigor, J. A., Van Toller, S., Behan, J., & Richardson, A. (1999). The effect of odour priming on long latency visual evoked potentials of matching and mismatching objects. *Chem Senses, 24*(2), 137-144. doi:DOI 10.1093/chemse/24.2.137

Guido, G. (2001). *The salience of marketing stimuli: An incongruity-salience hypothesis on consumer awareness*: Springer Science & Business Media.

Gur, R. C., Sara, R., Hagendoorn, M., Marom, O., Hughett, P., Macy, L., . . . Gur, R. E. (2002). A method for obtaining 3-dimensional facial expressions and its standardization for use in neurocognitive studies. *Journal of Neuroscience Methods, 115*(2), 137-143.

Hajcak, G., Dunning, J. P., & Foti, D. (2007). Neural response to emotional pictures is unaffected by concurrent task difficulty: an event-related potential study. *Behav Neurosci, 121*(6), 1156.

Hajcak, G., MacNamara, A., & Olvet, D. M. (2010). Event-related potentials, emotion, and emotion regulation: an integrative review. *Developmental Neuropsychology, 35*(2), 129-155.

Hajcak, G., Moser, J. S., & Simons, R. F. (2006). Attending to affect: appraisal strategies modulate the electrocortical response to arousing pictures. *Emotion, 6*(3), 517.

Hajcak, G., & Olvet, D. M. (2008). The persistence of attention to emotion: brain potentials during and after picture presentation. *Emotion, 8*(2), 250.

Hari, R., Parkkonen, L., & Nangini, C. (2010). The brain in time: insights from neuromagnetic recordings. *Ann N Y Acad Sci, 1191*, 89-109. doi:10.1111/j.1749-6632.2010.05438.x

Hawkes, C. (2003). Olfaction in neurodegenerative disorder. *Movement Disorders, 18*(4), 364-372.

Hendee, W. R., & Morgan, C. J. (1984). Magnetic resonance imaging. Part I--physical principles. *West J Med, 141*(4), 491-500.

Henkin, R. I., & Levy, L. M. (2001). Lateralization of brain activation to imagination and smell of odors using functional magnetic resonance imaging (fMRI): left hemispheric localization of pleasant and right hemispheric localization of unpleasant odors. *Journal of computer assisted tomography, 25*(4), 493-514. doi:10.1097/00004728-200107000-00001

Henrich, J., Heine, S. J., & Norenzayan, A. (2010). The weirdest people in the world? *Behav Brain Sci, 33*(2-3), 61-83. doi:doi:10.1017/S0140525X0999152X

Hermans, D., & Baeyens, F. (2002). Acquisition and activation of odor hedonics in everyday situations: Conditioning and priming studies. *Olfaction, taste, and cognition*, 119-139.

Hermans, D., Baeyens, F., & Eelen, P. (1998). Odours as affective-processing context for word evaluation: A case of cross-modal affective priming. *Cognition & Emotion, 12*(4), 601-613. doi:Doi 10.1080/026999398379583

Hermans, D. D. H., J. Eelen, P. (2001). A time course analysis of the affective priming effect *Cognition and Emotion, 15*(2), 143-165.

Herring, D. R., Taylor, J. H., White, K. R., & Crites Jr, S. L. (2011). Electrophysiological responses to evaluative priming: the LPP is sensitive to incongruity. *Emotion, 11*(4), 794. doi:10.1037/a0022804

Herring, D. R., White, K. R., Jabeen, L. N., Hinojos, M., Terrazas, G., Reyes, S. M., . . . Crites, S. L. (2013). On the automatic activation of attitudes: a quarter century of evaluative priming research. *Psychol Bull, 139*(5), 1062-1089. doi:10.1037/a0031309

Herrmann, C., Ziegler, S., Birbaumer, N., & Flor, H. (2000). Pavlovian aversive and appetitive odor conditionining in humans: subjective, peripheral, and electrocortical changes. . *Exp. Brain Res., 132*, 203-215. doi:10.1007/s002210000343

Herz, R. S., Eliassen, J., Beland, S., & Souza, T. (2004). Neuroimaging evidence for the emotional potency of odor-evoked memory. *Neuropsychologia, 42*(3), 371-378.

Herz, R. S., & Engen, T. (1996). Odor memory: Review and analysis. *Psychon Bull Rev, 3*(3), 300-313. doi:Doi 10.3758/Bf03210754

Hietanen, J. K., & Astikainen, P. (2013). N170 response to facial expressions is modulated by the affective congruency between the emotional expression and preceding affective picture. *Biol Psychol, 92*(2), 114-124. doi:http://dx.doi.org/10.1016/j.biopsycho.2012.10.005

Hofmann, W., De Houwer, J., Perugini, M., Baeyens, F., & Crombez, G. (2010). Evaluative conditioning in humans: a meta-analysis. *Psychol Bull, 136*(3), 390. doi:10.1037/a0018916

Homma, I., & Masaoka, Y. (2008). Breathing rhythms and emotions. *Exp Physiol, 93*(9), 1011-1021. doi:10.1113/expphysiol.2008.042424

Hopf, J.-M., Vogel, E., Woodman, G., Heinze, H.-J., & Luck, S. J. (2002). Localizing visual discrimination processes in time and space. *Journal of Neurophysiology, 88*(4), 2088-2095.

Hornak, J. P. (1996). The basics of MRI: Rochester Institute of Technology.

Howarth, C., Hutton, C., & Deichmann, R. (2006). Improvement of the image quality of T1-weighted anatomical brain scans. *Neuroimage, 29*(3), 930-937. doi:10.1016/j.neuroimage.2005.08.004

Huart, C., Legrain, V., Hummel, T., Rombaux, P., & Mouraux, A. (2012). Time-frequency analysis of chemosensory event-related potentials to characterize

the cortical representation of odors in humans. *PLoS One, 7*(3), e33221. doi:10.1371/journal.pone.0033221

Hummel, T., Sekinger, B., Wolf, S. R., Pauli, E., & Kobal, G. (1997). 'Sniffin'Sticks': Olfactory Performance Assessed by the Combined Testing of Odor Identification, Odor Discrimination and Olfactory Threshold. *Chem Senses, 22*(1), 39-52. doi:10.1093/chemse/22.1.39

Itier, R. J., & Taylor, M. J. (2004). N170 or N1? Spatiotemporal differences between object and face processing using ERPs. *Cerebral cortex, 14*(2), 132-142.

Ito, M. (1993). Movement and thought: identical control mechanisms by the cerebellum. *Trends in Neurosciences, 16*(11), 448-450. doi:http://dx.doi.org/10.1016/0166-2236(93)90073-U

Ito, M. (2008). Control of mental activities by internal models in the cerebellum. *Nat Rev Neurosci, 9*(4), 304-313.

Ito, T. A., Larsen, J. T., Smith, N. K., & Cacioppo, J. T. (1998). Negative information weighs more heavily on the brain: The negativity bias in evaluative categorizations. *J Pers Soc Psychol, 75*(4), 887-900. doi:10.1037/0022-3514.75.4.887

Jasper, H. (1958). Report of committee on methods of clinical exam in EEG. *Electroencephalogr Clin Neurophysiol, 10*, 370-375.

Jin, J., Zelano, C., Gottfried, J. A., & Mohanty, A. (2015). Human Amygdala Represents the Complete Spectrum of Subjective Valence. *J Neurosci, 35*(45), 15145-15156. doi:10.1523/jneurosci.2450-15.2015

Johnson, E. J., & Tversky, A. (1983). Affect, generalization, and the perception of risk. *J Pers Soc Psychol, 45*(1), 20.

Jones-Gotman, M., & Zatorre, R. J. (1988). Olfactory identification deficits in patients with focal cerebral excision. *Neuropsychologia, 26*(3), 387-400.

Jones-Gotman, M., & Zatorre, R. J. (1993). Odor recognition memory in humans: role of right temporal and orbitofrontal regions. *Brain Cogn, 22*(2), 182-198.

Jung, T. P., Makeig, S., Humphries, C., Lee, T. W., McKeown, M. J., Iragui, V., & Sejnowski, T. J. (2000). Removing electroencephalographic artifacts by blind source separation. *Psychophysiology, 37*(2), 163-178.

Kahneman, D., & Tversky, A. (2000). *Choices, values, and frames*: Cambridge University Press.

Kamp, A., Pfurtscheller, G., Edlinger, G., & Lopes da Silva, F. (2005). Technological basis of EEG recordings. In E. Niedermeyer (Ed.), *Electroencephalography: basic principles, clinical applications and related fields* (5th ed., pp. 127-138). Philadelphia, Pa. ; London: Lippincott Williams & Wilkins.

Kay, L. M., & Freeman, W. J. (1998). Bidirectional processing in the olfactory-limbic axis during olfactory behavior. *Behav Neurosci, 112*(3), 541-553.

Kiebel, S. J., & Friston, K. J. (2004). Statistical parametric mapping for event-related potentials (II): a hierarchical temporal model. *Neuroimage, 22*(2), 503-520.

Kim, S.-G., & Bandettini, P. A. (2010). Principles of functional MRI *BOLD fMRI* (pp. 3-22): Springer.

Kim, S. G., & Ogawa, S. (2012). Biophysical and physiological origins of blood oxygenation level-dependent fMRI signals. *J Cereb Blood Flow Metab, 32*(7), 1188-1206. doi:10.1038/jcbfm.2012.23

Kirk-Smith, M. D., Van Toller, C., & Dodd, G. H. (1983). Unconscious odour conditioning in human subjects. *Biol Psychol, 17*(2-3), 221-231.

Kirk-Smith, M. D. B., D. A. (1990). Effects of five odorants on mood and assessment of other people. In D. M.-S. D.W. Macdonald, S. Natynczuk (Ed.), *Chemical signals in vertebrates* (Vol. V, pp. 48-54). London: Oxford University Press.

Klem, G. H., Luders, H. O., Jasper, H. H., & Elger, C. (1999). The ten-twenty electrode system of the International Federation. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl, 52*, 3-6.

Knasko, S. C. (1992). Ambient odor's effect on creativity, mood, and perceived health. *Chemical senses, 17*(1), 27-35.

Knasko, S. C. (1995). Pleasant odors and congruency: effects on approach behavior. *Chemical senses, 20*(5), 479-487.

Kohler, C. G., Turner, T. H., Bilker, W. B., Brensinger, C. M., Siegel, S. J., Kanes, S. J., . . . Gur, R. C. (2003). Facial emotion recognition in schizophrenia: intensity effects and error pattern. *American Journal of Psychiatry, 160*(10), 1768-1774.

Kühn, S., & Gallinat, J. (2012). The neural correlates of subjective pleasantness. *Neuroimage, 61*(1), 289-294.

Kutas, M., & Federmeier, K. D. (2011). Thirty years and counting: finding meaning in the N400 component of the event-related brain potential (ERP). *Annu Rev Psychol, 62*, 621-647. doi:10.1146/annurev.psych.093008.131123

LaBar, K. S., & Cabeza, R. (2006). Cognitive neuroscience of emotional memory. *Nature Reviews Neuroscience, 7*(1), 54-64.

Lane, R. D., Reiman, E. M., Bradley, M. M., Lang, P. J., Ahern, G. L., Davidson, R. J., & Schwartz, G. E. (1997). Neuroanatomical correlates of pleasant and unpleasant emotion. *Neuropsychologia, 35*(11), 1437-1444. doi:10.1016/S0028-3932(97)00070-5

Lang, P. J., Bradley, M. M., Fitzsimmons, J. R., Cuthbert, B. N., Scott, J. D., Moulder, B., & Nangia, V. (1998). Emotional arousal and activation of the visual cortex: an fMRI analysis. *Psychophysiology, 35*(2), 199-210. doi:10.3109/00207454.2012.742894

Lawless, H. T., & Malone, G. J. (1986). COMPARISON OF RATING SCALES: SENSITIVITY, REPLICATES AND RELATIVE MEASUREMENT. *Journal of Sensory Studies, 1*(2), 155-174. doi:10.1111/j.1745-459X.1986.tb00168.x

Lebreton, M., Bertoux, M., Boutet, C., Lehericy, S., Dubois, B., Fossati, P., & Pessiglione, M. (2013). A critical role for the hippocampus in the valuation of imagined outcomes. *PLoS Biol, 11*(10), e1001684.

Lebreton, M., Jorge, S., Michel, V., Thirion, B., & Pessiglione, M. (2009). An automatic valuation system in the human brain: evidence from functional neuroimaging. *Neuron, 64*(3), 431-439.

LeDoux, J. E. (1993). Emotional memory systems in the brain. *Behavioural brain research, 58*(1-2), 69-79.

Lehmann, D. (1987). Principles of spatial analysis. In A. S. Gevins & A. Remond (Eds.), *Handbook of Electroencephalography and Clinical Neurophysiology: Methods of Analysis of Brain Electrical and Magnetic Signals* (Revised series ed., pp. 309-354). Amsterdam: Elsevier.

Leleu, A., Demily, C., Franck, N., Durand, K., Schaal, B., & Baudouin, J. Y. (2015). The Odor Context Facilitates the Perception of Low-Intensity Facial Expressions of Emotion. *PLoS One, 10*(9), e0138656. doi:10.1371/journal.pone.0138656

Leleu, A., Godard, O., Dollion, N., Durand, K., Schaal, B., & Baudouin, J. Y. (2015). Contextual odors modulate the visual processing of emotional facial expressions: An ERP study. *Neuropsychologia, 77*, 366-379. doi:10.1016/j.neuropsychologia.2015.09.014

Leppanen, J. M., & Hietanen, J. K. (2003). Affect and Face Perception: Odors Modulate the Recognition Advantage of Happy Faces. *Emotion, 3*(4), 315-326. doi:10.1037/1528-3542.3.4.315

Lerner, J. S., & Keltner, D. (2001). Fear, anger, and risk. *J Pers Soc Psychol, 81*(1), 146.

Levey, A. B., & Martin, I. (1975). Classical conditioning of human 'evaluative'responses. *Behaviour research and therapy, 13*(4), 221-226.

Levine, J. M., & McBurney, D. (1986). *The role of olfaction in social perception and behavior*: Learning Research and Development Center, University of Pittsburgh.

Levy, I., Lazzaro, S. C., Rutledge, R. B., & Glimcher, P. W. (2011). Choice from non-choice: predicting consumer preferences from blood oxygenation level-dependent signals obtained during passive viewing. *The Journal of Neuroscience, 31*(1), 118-125.

Li, W., Moallem, I., Paller, K. A., & Gottfried, J. A. (2007). Subliminal smells can guide social preferences. *Psychological Science, 18*(12), 1044-1049. doi:10.1111/j.1467-9280.2007.02023.x

Lim, J. (2011). Hedonic scaling: A review of methods and theory. *Food quality and preference, 22*(8), 733-747.

Lindquist, M. A. (2008). The statistical analysis of fMRI data. *Statistical Science, 23*(4), 439-464.

Lipton, M. L. (2010). *Totally accessible MRI: a user's guide to principles, technology, and applications*: Springer Science & Business Media.

Loewenstein, G., & Lerner, J. S. (2003). The role of affect in decision making. *Handbook of affective science, 619*(642), 3.

Logothetis, N. K., Pauls, J., Augath, M., Trinath, T., & Oeltermann, A. (2001). Neurophysiological investigation of the basis of the fMRI signal. *Nature, 412*(6843), 150-157. doi:10.1038/35084005

Lopes da Silva, F. (2005). Event-Related Potentials: Methodology and Quantification. In E. Niedermeyer (Ed.), *Electroencephalography: basic*

principles, clinical applications and related fields (5th ed., pp. 991-1002). Philadelphia, Pa. ; London: Lippincott Williams & Wilkins.

Lopes da Silva, F., & Van Rotterdam, A. (2005). Biophysical aspects of EEG and MEG generation. In E. Niedermeyer (Ed.), *Electroencephalography: basic principles, clinical applications and related fields* (5th ed., pp. 17-29). Philadelphia, Pa. ; London: Lippincott Williams & Wilkins.

Luck, S. J. (2005). *An introduction to the event-related potential technique*. Cambridge, Mass. ; London: MIT Press.

Luck, S. J., Woodman, G. F., & Vogel, E. K. (2000). Event-related potential studies of attention. *Trends in cognitive sciences, 4*(11), 432-440.

Macaluso, E., Frith, C. D., & Driver, J. (2000). Modulation of human visual cortex by crossmodal spatial attention. *Science, 289*(5482), 1206-1208.

Mackay-Sim, A., & Royet, J.-P. (2006). Structure and function of the olfactory system. *Olfaction and the brain*, 3-27.

MacNamara, A., & Hajcak, G. (2010). Distinct electrocortical and behavioral evidence for increased attention to threat in generalized anxiety disorder. *Depression and Anxiety, 27*(3), 234-243.

Mainland, J., & Sobel, N. (2006). The sniff is part of the olfactory percept. *Chemical senses, 31*(2), 181-196. doi:10.1093/chemse/bjj012

Mandal, M. K., Tandon, S. C., & Asthana, H. S. (1991). Right Brain Damage Impairs Recognition of Negative Emotions. *Cortex, 27*(2), 247-253. doi:10.1016/S0010-9452(13)80129-3

Mandeville, J. B., & Rosen, B. R. (2002). Functional MRI. In A. W. Toga & J. C. Mazziotta (Eds.), *Brain mapping* (2nd ed., pp. 315-349). Amsterdam ; London: Academic.

Maris, E., & Oostenveld, R. (2007). Nonparametric statistical testing of EEG-and MEG-data. *Journal of Neuroscience Methods, 164*(1), 177-190.

Markowitsch, H. J., Calabrese, P., Würker, M., Durwen, H. F., Kessler, J., Babinsky, R., . . . Gehlen, W. (1994). The amygdala's contribution to memory-a study on two patients with Urbach-Wiethe disease. *Neuroreport, 5*(11), 1349-1352.

Martin, I., & Levey, A. (1994). The evaluative response: Primitive but necessary. *Behaviour research and therapy, 32*(3), 301-305.

Masaoka, Y., Koiwa, N., & Homma, I. (2005). Inspiratory phase-locked alpha oscillation in human olfaction: source generators estimated by a dipole

tracing method. *J Physiol, 566*(Pt 3), 979-997. doi:10.1113/jphysiol.2005.086124

Matsumoto, D., & Ekman, P. (1988). *Japanese and Caucasian facial expressions of emotion and neutral faces*: els autors.

McCabe, C., & Rolls, E. T. (2007). Umami: a delicious flavor formed by convergence of taste and olfactory pathways in the human brain. *Eur J Neurosci, 25*(6), 1855-1864. doi:10.1111/j.1460-9568.2007.05445.x

McDonald, A. J. (1998). Cortical pathways to the mammalian amygdala. *Prog Neurobiol, 55*(3), 257-332.

McFadden, D. L. (2005). Revealed stochastic preference: a synthesis. *Economic Theory, 26*(2), 245-264.

McGlone, F., Österbauer, R. A., Demattè, L. M., & Spence, C. (2013). The Crossmodal Influence of Odor Hedonics on Facial Attractiveness: Behavioural and fMRI Measures. *Functional Brain Mapping and the Endeavor to Understand the Working Brain*(11), 209-225. doi:10.5772/56504

McRobbie, D. W., Moore, E. A., Graves, M. J., & Prince, M. R. (2007). *MRI from Picture to Proton*: Cambridge university press.

Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Structure and Function, 214*(5), 655-667. doi:10.1007/s00429-010-0262-0

Meredith, M. A., & Stein, B. E. (1983). Interactions among converging sensory inputs in the superior colliculus. *Science, 221*(4608), 389-391.

Mesholam, R. I., Moberg, P. J., Mahr, R. N., & Doty, R. L. (1998). Olfaction in neurodegenerative disease: a meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases. *Archives of neurology, 55*(1), 84-90.

Millot, J., & Brand, G. (2001). Effects of pleasant and unpleasant ambient odors on human voice pitch. *Neurosci Lett, 297*(1), 61-63.

Miltner, W., Matjak, M., Braun, C., Diekmann, H., & Brody, S. (1994). Emotional qualities of odors and their influence on the startle reflex in humans. *Psychophysiology, 31*(1), 107-110.

Mitchell, D. J., Kahn, B. E., & Knasko, S. C. (1995). There's Something in the Air: Effects of Congruent or Incongruent Ambient Odor on Consumer Decision Making. *Journal of Consumer Research, 22*(2), 229-238. doi:10.1086/209447

Murphy, C. (1999). Loss of olfactory function in dementing disease. *Physiology & behavior, 66*(2), 177-182.

Naatanen, R., & Picton, T. W. (1986). N2 and automatic versus controlled processes. *Electroencephalogr Clin Neurophysiol Suppl, 38*, 169-186.

Narashiman, P. T., & Jacobs, R. E. (2002). Neuroanatomical micromagnetic resonance imaging. In A. W. Toga & J. C. Mazziotta (Eds.), *Brain mapping* (2nd ed., pp. 399-426). Amsterdam ; London: Academic.

Nunez, P. L., & Silberstein, R. B. (2000). On the relationship of synaptic activity to macroscopic measurements: does co-registration of EEG with fMRI make sense? *Brain Topogr, 13*(2), 79-96.

Nunez, P. L., Srinivasan, R., Westdorp, A. F., Wijesinghe, R. S., Tucker, D. M., Silberstein, R. B., & Cadusch, P. J. (1997). EEG coherency. I: Statistics, reference electrode, volume conduction, Laplacians, cortical imaging, and interpretation at multiple scales. *Electroencephalogr Clin Neurophysiol, 103*(5), 499-515.

O'Doherty, J., Winston, J., Critchley, H., Perrett, D., Burt, D. M., & Dolan, R. J. (2003). Beauty in a smile: the role of medial orbitofrontal cortex in facial attractiveness. *Neuropsychologia, 41*(2), 147-155.

Ogawa, S., Lee, T.-M., Stepnoski, R., Chen, W., Zhu, X.-H., & Ugurbil, K. (2000). An approach to probe some neural systems interaction by functional MRI at neural time scale down to milliseconds. *Proc Natl Acad Sci U S A, 97*(20), 11026-11031. doi:10.1073/pnas.97.20.11026

Ohloff, G. (1994). *Scent and fragrances. The fascination of odors and their chemical perspectives*: Springer-Verlag.

Öhman, A. (2002). Automaticity and the amygdala: Nonconscious responses to emotional faces. *Current directions in psychological science, 11*(2), 62-66.

Olofsson, J. K., Bowman, N. E., Khatibi, K., & Gottfried, J. A. (2012). A time-based account of the perception of odor objects and valences. *Psychological Science, 23*(10), 1224-1232. doi:10.1177/0956797612441951

Ongur, D., Ferry, A. T., & Price, J. L. (2003). Architectonic subdivision of the human orbital and medial prefrontal cortex. *J Comp Neurol, 460*(3), 425-449. doi:10.1002/cne.10609

Ongur, D., & Price, J. L. (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cereb Cortex, 10*(3), 206-219.

Padoa-Schioppa, C. (2009). Range-Adapting Representation of Economic Value in the Orbitofrontal Cortex. *J Neurosci, 29*(44), 14004-14014. doi:10.1523/JNEUROSCI.3751-09.2009

Padoa-Schioppa, C., & Assad, J. A. (2006). Neurons in the orbitofrontal cortex encode economic value. *Nature, 441*(7090), 223-226. doi:10.1038/nature04676

Pause, B. M., Ohrt, A., Prehn, A., & Ferstl, R. (2004). Positive emotional priming of facial affect perception in females is diminished by chemosensory anxiety signals. *Chem Senses, 29*(9), 797-805. doi:10.1093/chemse/bjh245

Paustenbach, D. J., & Gaffney, S. H. (2006). The role of odor and irritation, as well as risk perception, in the setting of occupational exposure limits. *International archives of occupational and environmental health, 79*(4), 339-342.

Peeters, G., & Czapinski, J. (1990). Positive-negative asymmetry in evaluations: The distinction between affective and informational negativity effects. *European review of social psychology, 1*(1), 33-60.

Petrides, M., & Pandya, D. N. (2012). Chapter 26 - The Frontal Cortex A2 - Mai, Jürgen K. In G. Paxinos (Ed.), *The Human Nervous System (Third Edition)* (pp. 988-1011). San Diego: Academic Press.

Phelps, E. A. (2004). Human emotion and memory: interactions of the amygdala and hippocampal complex. *Current opinion in neurobiology, 14*(2), 198-202.

Phelps, E. A., & LeDoux, J. E. (2005). Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron, 48*(2), 175-187. doi:10.1016/j.neuron.2005.09.025

Phillips, M. L., Young, A. W., Senior, C., Brammer, M., Andrew, C., Calder, A. J., . . . Williams, S. (1997). A specific neural substrate for perceiving facial expressions of disgust. *Nature, 389*(6650), 495-498.

Platek, S. M., Thomson, J. W., & Gallup, G. G., Jr. (2004). Cross-modal self-recognition: the role of visual, auditory, and olfactory primes. *Conscious Cogn, 13*(1), 197-210. doi:10.1016/j.concog.2003.10.001

Polich, J. (2012). Neuropsychology of P300 *Oxford handbook of event-related potential components* (pp. 159-188).

Poline, J. B., Holmes, A. P., Worsley, K. J., & Friston, K. J. (1997). Statistical inference and the theory of random fields. *SPM short course notes*, 1-27.

Pollatos, O., Kopietz, R., Linn, J., Albrecht, J., Sakar, V., Anzinger, A., . . . Wiesmann, M. (2007). Emotional stimulation alters olfactory sensitivity and odor judgment. *Chem Senses, 32*(6), 583-589. doi:10.1093/chemse/bjm027

Pooley, R. A. (2005). Fundamental Physics of MR Imaging 1. *Radiographics, 25*(4), 1087-1099.

Potter, H., & Butters, N. (1980). An assessment of olfactory deficits in patients with damage to prefrontal cortex. *Neuropsychologia, 18*(6), 621-628.

Price, J. L. (1985). Beyond the primary olfactory cortex: olfactory-related areas in the neocortex, thalamus and hypothalamus. *Chemical senses, 10*(2), 239-258.

Raghunathan, R., & Pham, M. T. (1999). All Negative Moods Are Not Equal: Motivational Influences of Anxiety and Sadness on Decision Making. *Organizational Behavior and Human Decision Processes, 79*(1), 56-77. doi:http://dx.doi.org/10.1006/obhd.1999.2838

Rees, G., Friston, K., & Koch, C. (2000). A direct quantitative relationship between the functional properties of human and macaque V5. *Nat Neurosci, 3*(7), 716-723. doi:10.1038/76673

Richardson, J. T., & Zucco, G. M. (1989). Cognition and olfaction: a review. *Psychol Bull, 105*(3), 352-360.

Righart, R., & de Gelder, B. (2006). Context influences early perceptual analysis of faces--an electrophysiological study. *Cereb Cortex, 16*(9), 1249-1257. doi:10.1093/cercor/bhj066

Ritter, W., Simson, R., & Vaughan, H. G., Jr. (1983). Event-related potential correlates of two stages of information processing in physical and semantic discrimination tasks. *Psychophysiology, 20*(2), 168-179.

Ritz, T., George, C., & Dahme, B. (2000). Respiratory resistance during emotional stimulation: evidence for a nonspecific effect of experienced arousal? *Biol Psychol, 52*(2), 143-160. doi:http://dx.doi.org/10.1016/S0301-0511(99)00026-5

Robinson, A. K., Reinhard, J., & Mattingley, J. B. (2014). Olfaction Modulates Early Neural Responses to Matching Visual Objects. *Journal of cognitive neuroscience, 27*(4), 832-841. doi:10.1162/jocn_a_00732

Rolls, E. T. (2000). The orbitofrontal cortex and reward. *Cerebral cortex, 10*(3), 284-294.

Rolls, E. T. (2004a). The functions of the orbitofrontal cortex. *Brain Cogn, 55*(1), 11-29.

Rolls, E. T. (2004b). Smell, taste, texture, and temperature multimodal representations in the brain, and their relevance to the control of appetite. *Nutr Rev, 62*(11 Pt 2), S193-204; discussion S224-141.

Rolls, E. T., & Baylis, L. L. (1994). Gustatory, olfactory, and visual convergence within the primate orbitofrontal cortex. *The Journal of Neuroscience, 14*(9), 5437-5452.

Rolls, E. T., Critchley, H. D., & Treves, A. (1996). Representation of olfactory information in the primate orbitofrontal cortex. *Journal of Neurophysiology, 75*(5), 1982-1996.

Rolls, E. T., & Grabenhorst, F. (2008). The orbitofrontal cortex and beyond: from affect to decision-making. *Prog Neurobiol, 86*(3), 216-244. doi:10.1016/j.pneurobio.2008.09.001

Rolls, E. T., Grabenhorst, F., Margot, C., da Silva, M. A., & Velazco, M. I. (2008). Selective attention to affective value alters how the brain processes olfactory stimuli. *J Cogn Neurosci, 20*(10), 1815-1826. doi:10.1162/jocn.2008.20128

Rolls, E. T., Grabenhorst, F., & Parris, B. A. (2010). Neural systems underlying decisions about affective odors. *J Cogn Neurosci, 22*(5), 1069-1082. doi:10.1162/jocn.2009.21231

Rolls, E. T., Kringelbach, M. L., & de Araujo, I. E. (2003). Different representations of pleasant and unpleasant odours in the human brain. *Eur J Neurosci, 18*(3), 695-703.

Rossion, B., Dricot, L., Devolder, A., Bodart, J.-M., Crommelinck, M., De Gelder, B., & Zoontjes, R. (2000). Hemispheric asymmetries for whole-based and part-based face processing in the human fusiform gyrus. *Journal of cognitive neuroscience, 12*(5), 793-802.

Rossion, B., Gauthier, I., Tarr, M. J., Despland, P., Bruyer, R., Linotte, S., & Crommelinck, M. (2000). The N170 occipito-temporal component is delayed

and enhanced to inverted faces but not to inverted objects: an electrophysiological account of face-specific processes in the human brain. *Neuroreport, 11*(1), 69-72.

Rossion, B., & Jacques, C. (2011). 5 The N170: Understanding the Time Course. *The Oxford handbook of event-related potential components*, 115.

Rotton, J., Barry, T., Frey, J., & Soler, E. (1978). Air Pollution and interpersonal Attraction1. *Journal of Applied Social Psychology, 8*(1), 57-71.

Rowan, A. J., & Tolunsky, E. (2003). *A Primer of EEG: With a Mini-atlas*: Butterworth-Heinemann Medical.

Royet, J. P., Hudry, J., Zald, D. H., Godinot, D., Grégoire, M. C., Lavenne, F., . . . Holley, A. (2001). Functional neuroanatomy of different olfactory judgments. *Neuroimage, 13*(3), 506-519.

Royet, J. P., Koenig, O., Gregoire, M.-C., Cinotti, L., Lavenne, F., Le Bars, D., . . . Sicard, G. (1999). Functional anatomy of perceptual and semantic processing for odors. *Journal of cognitive neuroscience, 11*(1), 94-109.

Royet, J. P., & Plailly, J. (2004). Lateralization of olfactory processes. *Chemical senses, 29*(8), 731-745.

Royet, J. P., Plailly, J., Delon-Martin, C., Kareken, D. A., & Segebarth, C. (2003). fMRI of emotional responses to odors:: influence of hedonic valence and judgment, handedness, and gender. *Neuroimage, 20*(2), 713-728.

Royet, J. P., Zald, D., Versace, R., Costes, N., Lavenne, F., Koenig, O., & Gervais, R. (2000). Emotional responses to pleasant and unpleasant olfactory, visual, and auditory stimuli: a positron emission tomography study. *J Neurosci, 20*(20), 7752-7759.

Rubin, D., Botanov, Y., Hajcak, G., & Mujica-Parodi, L. R. (2012). Second-hand stress: inhalation of stress sweat enhances neural response to neutral faces. *Soc Cogn Affect Neurosci, 7*(2), 208-212. doi:10.1093/scan/nsq097

Rushworth, M. F. S., Kolling, N., Sallet, J., & Mars, R. B. (2012). Valuation and decision-making in frontal cortex: one or many serial or parallel systems? *Current opinion in neurobiology, 22*(6), 946-955. doi:http://dx.doi.org/10.1016/j.conb.2012.04.011

Sarfarazi, M., Cave, B., Richardson, A., Behan, J., & Sedgwick, E. M. (1999). Visual event related potentials modulated by contextually relevant and irrelevant olfactory primes. *Chem Senses, 24*(2), 145-154.

Sato, W., Yoshikawa, S., Kochiyama, T., & Matsumura, M. (2004). The amygdala processes the emotional significance of facial expressions: an fMRI investigation using the interaction between expression and face direction. *Neuroimage, 22*(2), 1006-1013. doi:10.1016/j.neuroimage.2004.02.030

Savic, I., Gulyas, B., Larsson, M., & Roland, P. (2000). Olfactory Functions Are Mediated by Parallel and Hierarchical Processing. *Neuron, 26*(3), 735-745. doi:http://dx.doi.org/10.1016/S0896-6273(00)81209-X

Schiffman, S. S., Sattely-Miller, E. A., Suggs, M. S., & Graham, B. G. (1995). The effect of pleasant odors and hormone status on mood of women at midlife. *Brain Res Bull, 36*(1), 19-29.

Schiffman, S. S., Suggs, M. S., & Sattely-Miller, E. A. (1995). Effect of pleasant odors on mood of males at midlife: comparison of African-American and European-American men. *Brain Res Bull, 36*(1), 31-37.

Schild, H. H. (1992). *MRI Made Easy:(--well Almost)*: Berlex Laboratories.

Schneider, S., & Strüder, H. K. (2012). EEG: Theoretical Background and Practical AspectsFunctional neuroimaging in exercise and sport sciences [electronic resource] (pp. 197-212). New York, NY: Springer. Retrieved from http://dx.doi.org/10.1007/978-1-4614-3293-7.

Seigneuric, A., Durand, K., Jiang, T., Baudouin, J. Y., & Schaal, B. (2010). The nose tells it to the eyes: crossmodal associations between olfaction and vision. *Perception, 39*(11), 1541-1554.

Seo, H. S., Arshamian, A., Schemmer, K., Scheer, I., Sander, T., Ritter, G., & Hummel, T. (2010). Cross-modal integration between odors and abstract symbols. *Neurosci Lett, 478*(3), 175-178. doi:10.1016/j.neulet.2010.05.011

Seo, H. S., & Hummel, T. (2011). Auditory-olfactory integration: congruent or pleasant sounds amplify odor pleasantness. *Chem Senses, 36*(3), 301-309. doi:10.1093/chemse/bjq129

Seo, H. S., Roidl, E., Muller, F., & Negoias, S. (2010). Odors enhance visual attention to congruent objects. *Appetite, 54*(3), 544-549. doi:10.1016/j.appet.2010.02.011

Sergent, J., Ohta, S., & MacDonald, B. (1992). Functional neuroanatomy of face and object processing. A positron emission tomography study. *Brain, 115 Pt 1*, 15-36.

Seubert, J., Freiherr, J., Djordjevic, J., & Lundstrom, J. N. (2012). Statistical localization of human olfactory cortex. *Neuroimage, 66C*, 333-342. doi:10.1016/j.neuroimage.2012.10.030

Seubert, J., Gregory, K. M., Chamberland, J., Dessirier, J., & Lundström, J. N. (2014). Odor Valence Linearly Modulates Attractiveness, but Not Age Assessment, of Invariant Facial Features in a Memory-Based Rating Task. *PLoS One, 9*(5), e98347. doi:10.1371/journal.pone.0098347

Seubert, J., Kellermann, T., Loughead, J., Boers, F., Brensinger, C., Schneider, F., & Habel, U. (2010). Processing of disgusted faces is facilitated by odor primes: a functional MRI study. *Neuroimage, 53*(2), 746-756. doi:10.1016/j.neuroimage.2010.07.012

Shepherd, G. M. (2005). Perception without a Thalamus: How Does Olfaction Do It? *Neuron, 46*(2), 166-168. doi:http://dx.doi.org/10.1016/j.neuron.2005.03.012

Small, D. M., Voss, J., Mak, Y. E., Simmons, K. B., Parrish, T., & Gitelman, D. (2004). Experience-dependent neural integration of taste and smell in the human brain. *Journal of Neurophysiology, 92*(3), 1892-1903.

Smith, N. K., Cacioppo, J. T., Larsen, J. T., & Chartrand, T. L. (2003). May I have your attention, please: Electrocortical responses to positive and negative stimuli. *Neuropsychologia, 41*(2), 171-183. doi:http://dx.doi.org/10.1016/S0028-3932(02)00147-1

Smith, N. K., Larsen, J. T., Chartrand, T. L., Cacioppo, J. T., Katafiasz, H. A., & Moran, K. E. (2006). Being bad isn't always good: Affective context moderates the attention bias toward negative information. *J Pers Soc Psychol, 90*(2), 210-220. doi:10.1037/0022-3514.90.2.210

Sobel, N., Prabhakaran, V., Desmond, J. E., Glover, G. H., Goode, R. L., Sullivan, E. V., & Gabrieli, J. D. (1998). Sniffing and smelling: separate subsystems in the human olfactory cortex. *Nature, 392*(6673), 282-286. doi:10.1038/32654

Sobel, N., Prabhakaran, V., Zhao, Z., Desmond, J. E., Glover, G. H., Sullivan, E. V., & Gabrieli, J. D. E. (2000). Time course of odorant-induced activation in the human primary olfactory cortex. *Journal of Neurophysiology, 83*(1), 537-551.

Sokol-Hessner, P., Hsu, M., Curley, N. G., Delgado, M. R., Camerer, C. F., & Phelps, E. A. (2009). Thinking like a trader selectively reduces individuals' loss aversion. *Proceedings of the National Academy of Sciences, 106*(13), 5035-5040.

Spangenberg, E. R., Crowley, A. E., & Henderson, P. W. (1996). Improving the store environment: do olfactory cues affect evaluations and behaviors? *The Journal of Marketing*, 67-80.

Speckmann, E.-J., Caspers, H., & Andersen, P. (1979). *Origin of cerebral field potential : international symposium, Muenster, Germany [October 10-11, 1977]*. Stuttgart: Thieme.

Speckmann, E. J., & Elger, C. (2005). Introduction to the neurophysiological basis of the EEG and DC potentials. In E. Niedermeyer (Ed.), *Electroencephalography: basic principles, clinical applications and related fields* (5th ed., pp. 17-29). Philadelphia, Pa. ; London: Lippincott Williams & Wilkins.

Squire, L. R., & Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science, 253*(5026), 1380-1386.

Stancak, A., Xie, Y., Fallon, N., Bulsing, P., Giesbrecht, T., Thomas, A., & Pantelous, A. A. (2015). Unpleasant odors increase aversion to monetary losses. *Biol Psychol, 107*, 1-9. doi:10.1016/j.biopsycho.2015.02.006

Stanford, T. R., & Stein, B. E. (2007). Superadditivity in multisensory integration: putting the computation in context. *Neuroreport, 18*(8), 787-792.

Stein, B. E., & Stanford, T. R. (2008). Multisensory integration: current issues from the perspective of the single neuron. *Nat Rev Neurosci, 9*(4), 255-266. doi:10.1038/nrn2331

Steinberg, C., Dobel, C., Schupp, H. T., Kissler, J., Elling, L., Pantev, C., & Junghofer, M. (2012). Rapid and highly resolving: affective evaluation of olfactorily conditioned faces. *J Cogn Neurosci, 24*(1), 17-27. doi:10.1162/jocn_a_00067

Stevenson, R. J. (2010). An initial evaluation of the functions of human olfaction. *Chem Senses, 35*(1), 3-20. doi:10.1093/chemse/bjp083

Takagi, S. (1989). *Human olfaction*: University of Tokyo Press Tokyo.

Takahashi, H., Fujie, S., Camerer, C., Arakawa, R., Takano, H., Kodaka, F., . . . Takemura, K. (2013). Norepinephrine in the brain is associated with aversion to financial loss. *Molecular psychiatry, 18*(1), 3-4.

Tanabe, T., Yarita, H., Iino, M., Ooshima, Y., & Takagi, S. (1975). An olfactory projection area in orbitofrontal cortex of the monkey. *Journal of Neurophysiology, 38*(5), 1269-1283.

Taylor, S. E. (1991). Asymmetrical effects of positive and negative events: the mobilization-minimization hypothesis. *Psychol Bull, 110*(1), 67.

Teplan, M. (2002). Fundamentals of EEG measurement. *Measurement science review, 2*(2), 1-11.

Todrank, J., Byrnes, D., Wrzesniewski, A., & Rozin, P. (1995). Odors Can Change Preferences for People in Photographs - a Cross-Modal Evaluative Conditioning Study with Olfactory Uss and Visual Css. *Learning and Motivation, 26*(2), 116-140. doi:10.1016/0023-9690(95)90001-2

Tottenham, N., Tanaka, J. W., Leon, A. C., McCarry, T., Nurse, M., Hare, T. A., . . . Nelson, C. (2009). The NimStim set of facial expressions: Judgments from untrained research participants. *Psychiatry Res, 168*(3), 242-249. doi:10.1016/j.psychres.2008.05.006

Tremblay, L., & Schultz, W. (1999). Relative reward preference in primate orbitofrontal cortex. *Nature, 398*(6729), 704-708. doi:10.1038/19525

Tucker, D. M. (1981). Lateral brain function, emotion, and conceptualization. *Psychol Bull, 89*(1), 19. doi:10.1037/0033-2909.89.1.19

Tversky, A., & Kahneman, D. (1991). Loss aversion in riskless choice: A reference-dependent model. *The quarterly journal of economics*, 1039-1061.

Tversky, A., & Kahneman, D. (1992). Advances in prospect theory: Cumulative representation of uncertainty. *Journal of Risk and uncertainty, 5*(4), 297-323.

Van Hoesen, G. W., Augustinack, J. C., Dierking, J., Redman, S. J., & Thangavel, R. (2000). The Parahippocampal Gyrus in Alzheimer's Disease: Clinical and Preclinical Neuroanatomical Correlates. *Ann N Y Acad Sci, 911*(1), 254-274. doi:10.1111/j.1749-6632.2000.tb06731.x

van Reekum, C. M., vann de Berg, H., & Frijda, N. H. (1999). Cross-modal preference acquisition: Evaluative conditioning of pictures by affective olfactory and auditory cues. *Cognition & Emotion, 13*(6), 831-836.

Vandenberghe, R., Price, C., Wise, R., Josephs, O., & Frackowiak, R. S. (1996). Functional anatomy of a common semantic system for words and pictures. *Nature, 383*(6597), 254-256. doi:10.1038/383254a0

Villemure, C., Slotnick, B. M., & Bushnell, M. C. (2003). Effects of odors on pain perception: deciphering the roles of emotion and attention. *Pain, 106*(1), 101-108.

Vrana, S. R., Spence, E. L., & Lang, P. J. (1988). The startle probe response: a new measure of emotion? *Journal of abnormal psychology*, *97*(4), 487.

Wager, T. D., Phan, K. L., Liberzon, I., & Taylor, S. F. (2003). Valence, gender, and lateralization of functional brain anatomy in emotion: a meta-analysis of findings from neuroimaging. *Neuroimage*, *19*(3), 513-531. doi:http://dx.doi.org/10.1016/S1053-8119(03)00078-8

Walla, P. (2008). Olfaction and its dynamic influence on word and face processing: cross-modal integration. *Prog Neurobiol*, *84*(2), 192-209. doi:10.1016/j.pneurobio.2007.10.005

Walla, P., & Deecke, L. (2010). Odours Influence Visually Induced Emotion: Behavior and Neuroimaging. *Sensors (Basel, Switzerland)*, *10*(9), 8185-8197. doi:10.3390/s100908185

Walla, P., Mayer, D., Deecke, L., & Lang, W. (2005). How chemical information processing interferes with face processing: a magnetoencephalographic study. *Neuroimage*, *24*(1), 111-117. doi:10.1016/j.neuroimage.2004.09.030

Weinberg, A., & Hajcak, G. (2010). Beyond good and evil: the time-course of neural activity elicited by specific picture content. *Emotion*, *10*(6), 767.

Whitney, C., Kirk, M., O'Sullivan, J., Lambon Ralph, M. A., & Jefferies, E. (2010). The Neural Organization of Semantic Control: TMS Evidence for a Distributed Network in Left Inferior Frontal and Posterior Middle Temporal Gyrus. *Cerebral cortex*. doi:10.1093/cercor/bhq180

Wicker, B., Keysers, C., Plailly, J., Royet, J.-P., Gallese, V., & Rizzolatti, G. (2003). Both of Us Disgusted in My Insula: The Common Neural Basis of Seeing and Feeling Disgust. *Neuron*, *40*(3), 655-664. doi:http://dx.doi.org/10.1016/S0896-6273(03)00679-2

Winston, J. S., Gottfried, J. A., Kilner, J. M., & Dolan, R. J. (2005). Integrated neural representations of odor intensity and affective valence in human amygdala. *The Journal of Neuroscience*, *25*(39), 8903-8907.

Worsley, K. J. (2003). Developments in random field theory. *Hum. Brain. Func.*, *2*, 881-886.

Wright, H., Li, X., Fallon, N. B., Giesbrecht, T., Thomas, A., Harrold, J. A., . . . Stancak, A. (2015). Heightened eating drive and visual food stimuli attenuate central nociceptive processing. *J Neurophysiol*, *113*(5), 1323-1333. doi:10.1152/jn.00504.2014

Zald, D. H. (2003). The human amygdala and the emotional evaluation of sensory stimuli. *Brain Res Brain Res Rev, 41*(1), 88-123.

Zald, D. H., & Pardo, J. V. (1997). Emotion, olfaction, and the human amygdala: amygdala activation during aversive olfactory stimulation. *Proceedings of the National Academy of Sciences, 94*(8), 4119-4124.

Zald, D. H., & Pardo, J. V. (2000). Functional neuroimaging of the olfactory system in humans. *International Journal of Psychophysiology, 36*(2), 165-181.

Zatorre, R. J., Jones-Gotman, M., Evans, A. C., & Meyer, E. (1992). Functional localization and lateralization of human olfactory cortex. *Nature, 360*, 339-340.

Zatorre, R. J., Jones-Gotman, M., & Rouby, C. (2000). Neural mechanisms involved in odor pleasantness and intensity judgments. *Neuroreport, 11*(12), 2711-2716.

Zeki, S., Watson, J. D., Lueck, C. J., Friston, K. J., Kennard, C., & Frackowiak, R. S. (1991). A direct demonstration of functional specialization in human visual cortex. *J Neurosci, 11*(3), 641-649.

Zhang, Q., Lawson, A., Guo, C., & Jiang, Y. (2006). Electrophysiological correlates of visual affective priming. *Brain Res Bull, 71*(1-3), 316-323. doi:10.1016/j.brainresbull.2006.09.023

Zhang, Q., Li, X., Gold, B. T., & Jiang, Y. (2010). Neural correlates of cross-domain affective priming. *Brain research, 1329*, 142-151. doi:10.1016/j.brainres.2010.03.021

Zhou, W., & Chen, D. (2009). Fear-related chemosignals modulate recognition of fear in ambiguous facial expressions. *Psychological Science, 20*(2), 177-183. doi:10.1111/j.1467-9280.2009.02263.x

Appendices

Appendix 1: Sample ethics application form

UNIVERSITY OF LIVERPOOL

COMMITTEE ON RESEARCH ETHICS

APPLICATION FOR APPROVAL OF A PROJECT INVOLVING HUMAN PARTICIPANTS, HUMAN DATA, OR HUMAN MATERIAL

NOTES

1) This application form is to be used by researchers seeking research ethics approval from the University, as per the University's Policy on Research Ethics involving Human Participation. If an application qualifies for expedited review (Section C) it may be reviewed at Level 2, by your School or Institute's research ethics process.

2) Applications to the University Research Ethics Committees must normally include an **application form, participant information sheet and consent form** (all templates available online), along with any other relevant information, and should be submitted by email to the relevant contact listed at http://www.liv.ac.uk/researchethics/apply,for,research,ethics/.

3) Applications from Student investigators: the Committee will require proof that your Supervisor has approved the application to be submitted. Please attach this to your email. Your supervisor must be copied in on all correspondence relating to your application.

4) This form must be completed by following the guidance notes, accessible at www.liv.ac.uk/researchethics. Please complete every section, using N/A if appropriate. Incomplete forms will be returned to the applicant.

5) For studies involving overseas sites, please ensure you have researched any local approvals that might be required. Wherever possible this should include local research ethics approval. In the absence of a research ethics approval body, other relevant local approvals should be obtained, e.g. authorisation from a site, letter from a local organisation or group etc.

6) This form does not constitute insurance approval which must be sought separately. Please contact the **University's Insurance and Risk Manager** if your project involves overseas sites, vulnerable groups or is a clinical trial.

7) Staff investigators: You are encouraged to discuss your proposal with your Head of Department prior to submitting for research ethics approval.

RESEARCH MUST NOT BEGIN UNTIL ETHICAL APPROVAL HAS BEEN OBTAINED

FAILURE TO SEEK RESEARCH ETHICS APPROVAL IS TAKEN EXTERMELY SERIOUSLY BY THE INSTITUTION.

BEFORE COMPLETING YOUR APPLICATION PLEASE CONFIRM WHAT APPROVAL YOU ARE SEEKING (please check with "x"):

a) Expedited review of an individual research project .....X.....

b) Full committee review of an individual research project .....

c) Committee review generic* approval .....

*to cover a cohort of projects using similar methodologies and in line with Policy on Generic Approvals which can be found at www.liv.ac.uk/researchethics . Boundaries of the research must be defined clearly. Approval may be granted for up to 3 years and will be subject to annual review.

Declaration of the:

Principal Investigator _____ **OR** **Supervisor and Student Investigator** ___x_____
(please check with a "x")

- The information in this form is accurate to the best of my knowledge and belief, and I take full responsibility for it.
- I have read and understand the University's Policy on Research Ethics
- I undertake to abide by the ethical principles underlying the Declaration of Helsinki and the University's good practice guidelines on the proper conduct of research, together with the codes of practice laid down by any relevant professional or learned society.
- If the research is approved, I undertake to adhere to the study plan, the terms of the full application of which the REC has given a favourable opinion, and any conditions set out by the REC in giving its favourable opinion.
- I undertake to seek an ethical opinion from the REC before implementing substantial amendments to the study plan or to the terms of the full application of which the REC has given a favourable opinion.
- I understand that I am responsible for monitoring the research at all times.
- If there are any serious adverse events, I understand that I am responsible for immediately stopping the research and alerting the Research Ethics Committee within 24 hours of the occurrence, via ethics@liv.ac.uk.
- I am aware of my responsibility to be up to date and comply with the requirements of the law and relevant guidelines relating to security and confidentiality of personal data.
- I understand that research records/data may be subject to inspection for audit purposes if required in future.
- I understand that personal data about me as a researcher in this application will be held by the University and that this will be managed according to the principles established in the Data Protection Act.
- I understand that the information contained in this application, any supporting documentation and all correspondence with the Research Ethics Committee relating to the application, will be subject to the provisions of the Freedom of Information Acts. The information may be disclosed in response to requests made under the Acts except where statutory exemptions apply.
- I understand that all conditions apply to any co-applicants and researchers involved in the study, and that it is my responsibility to ensure that they abide by them.
- **For Supervisors**: I understand my responsibilities as supervisor, and will ensure, to the best of my abilities, that the student investigator abides by the University's Policy on Research Ethics at all times.
- **For the Student Investigator**: I understand my responsibilities to work within a set of safety, ethical and other guidelines as agreed in advance with my supervisor and understand that I must comply with the University's regulations and any other applicable code of ethics at all times.

Signature of Supervisor : .....
DR ANDREJ STANCAK .....
Date: (26/02/2014)
Print Name:

Signature of Student Investigator: .....MISS STEPHANIE COOK.....
Date: (26/02/2014)
Print Name:

SECTION A - IDENTIFYING INFORMATION

A1) Title of the research *(PLEASE INCLUDE A SHORT LAY TITLE IN BRACKETS).*

The effects of pleasant and unpleasant odours on evaluations of neutral male and female faces: an EEG study.

A2) PRINCIPAL INVESTIGATOR

Title:	Dr.	**Staff number:**	406447
Forename/Initials:	Andrej	**Surname:**	Stancak
Post:	Senior lecturer	**Department:**	Psychological Sciences
Telephone:	01517946959	**E-mail:**	a.stancak@liv.ac.uk

A3) Student Investigator(s)

Title and Name	Post / Current programme (if student investigator)	Department/ School/Institution if not UoL	Phone	Email
Miss Stephanie Cook	Post grad Student	Psychology	01517946956	s.cook2@liv.ac.uk
Miss Hazel Wright	Post grad Student	Psychology	01517946956	hwright@liverpool.ac.uk

A4) Co-Applicants

Title and Name	Post / Current programme (if student investigator)	Department/ School/Institution if not UoL	Phone	Email
Dr Nicholas Fallon	Research Associate	Psychology	01517946951	nickfal@liverpool.ac.uk

SECTION B - PROJECT DETAILS

B1) **Proposed study dates and duration** (RESEARCH **MUST NOT BEGIN** UNTIL ETHICAL APPROVAL HAS BEEN OBTAINED)

Please complete as appropriate:

EITHER

a) **Starting as soon as ethical approval has been obtained**

YES *(PLEASE DELETE AS APPLICABLE)*

Approximate end date:	October 30, 2014

OR

b) Approximate dates:

Start date:		**End date**:	

B2) **Give a FULL LAY SUMMARY of the purpose, design and methodology of the planned research.** *N.B. Please use as little jargon or technical language as possible. Where jargon / technical language is unavoidable, please ensure you provide a lay explanation. Please define any acronyms. The summary must be understood by persons outside of the subject area including members of the general public*

The research will address the question of how low intensity pleasant and unpleasant odours affect hedonic evaluations of neutral male and female faces, whilst recording changes in oscillatory activity and electrical potentials seen on the surface of the scalp using electroencephalographic (EEG) recordings.

Odours and emotions are very closely linked, and the cross-modal effects of odours on other types of perception (e.g. visual) are well documented. Research has suggested that pleasant and unpleasant odours can modulate preferences of neutral objects. More specifically, a few studies have indicated that evaluative ratings of neutral faces can be shifted in the direction of the odour pleasantness (i.e. a pleasant odour will make someone rate a neutral face as more pleasant, with unpleasant odours having the opposite effect). However, these previous studies exhibit methodological flaws (in terms of outdated stimulus control and analysis methods) which can now be surpassed using modern techniques. Moreover, given the limited amount of studies investigating these odour 'priming' effects, an optimal paradigm for observing these effects has not been established. Nor have the effects been explored using a modern EEG system. This study will be the first to systematically study the effects of pleasant and unpleasant odours on judgements of neutral male and female faces using a modern, 129-channel EEG system. We aim to evaluate changes in brain potentials and oscillations using a cutting edge approach, to gain a better insight into the neural processes underlying the effects of odours on evaluation.

The proposed research consists of both a pilot experiment (for which 30 participants will be required) and a full EEG experiment (for which 20 different participants will be required). Prior to

taking part in either the pilot experiment or the full experiment, participants will be screened using a set of 12 familiar odours (e.g. orange, mint, coffee) to ensure that they are able to accurately discriminate between odours. Screening of olfactory sensitivity will be performed using 'Sniffin Stick' (Burghart, Germany) test prior to the experiment in room 2.04b in the Eleanor Rathbone Building and should last no more than 5 minutes. The test involves presenting 12 odour pens approximately 3 cm beneath a participant's nostrils. Participants are asked to identify each of the 12 test odours from a selection of 4 labels for each. Nine correct detections (out of 12 probes) are required to confirm normal sense of smell. During screening the participants will also be asked whether they suffer from any neurological disorders such as epilepsy, olfactory disorders such as hyposmia or anosmia, or breathing disorders such as asthma. Any participants reporting such problems will be informed that, as the research involves delivering odours whilst monitoring brain activity, they would not be suitable to take part. Participants will also be requested to self report their age, the reason for excluding participants over 35 will be explained to those who fall outside the boundary (this is the age when we begin to see a reduction in the number of olfactory receptors, i.e. sense of smell may begin to deteriorate slightly).

Following the olfactory sensitivity test, during the screening session the participants will also be asked to rate a set of 36 neutral male and female faces (the same set that will be used in the full experiment, in order to gather baseline ratings for each face). They will rate the faces using a simple computer task which presents the face and then prompts participants to rate the pleasantness of the face. This should take no more than 5 minutes.

The pilot experiment will take place in a single session comprising 108 trials (split into 3 blocks of 36 trials) in the EEG lab on the ground floor of the Eleanor Rathbone building. The duration of each block will be approximately 12 minutes and the entire session will last approximately 1 hour. This time will include 15 minutes for the initial set up of the equipment, 40 minutes for the completion of three experimental blocks, and 5 minutes for removal of equipment and debrief.

During each trial of the pilot experiment, a low intensity odour (e.g. jasmine, rotten cabbage) will be administered for 3 seconds using a custom built olfactometer (Dancer design, UK Ltd.) at a rate of 2.2 l/minute. The timing of the onset of odours will be synchronised to participant's inspirations manually using real-time respiratory data. In half of the trials in each block, each odour presentation will be followed by a 1 second rest period. After this, a neutral male or female face will appear on screen (on a monitor positioned approximately 1 metre in front of the participant) for 300 ms (NON-OVERLAP CONDITION). In the other half of the trials, there will be an overlap between odour and face presentation such that the odour will be presented for 3 seconds, and the neutral male or female face will be presented during the final 300 ms of this 3 second window (OVERLAP CONDITION). In both conditions, a visual analogue scale prompting participants to rate the pleasantness of the face will appear following face presentation. Once participants have rated the pleasantness of the face using a computer mouse in their right hand, a second scale prompting participants to rate the intensity of the odour that was presented will appear. Participants will indicate their perceived intensity of the perceived odour. There will then be a period of around 5 seconds before the onset of the next trial. Please note that the odours administered will be of very low concentrations, not capable of producing nausea or discomfort.

The full experiment will take place in a single session comprising 108 trials (split into 3 blocks of 36 trials), in the EEG lab on the ground floor of the Eleanor Rathbone building. The duration of each block will be approximately 12 minutes and the entire session will last approximately 1 hour 15 minutes. This time will include 15 minutes for the initial set up of equipment, 50 minutes for the completion of three experimental blocks, and 10 minutes for removal of equipment and de-brief. Depending on results of the pilot experiment, the procedure for each trial will follow either

that of the overlap condition or the non-overlap condition (see above). All other details will remain the same except for that EEG will also be recorded.

During the full experiment, brain potentials will be recorded using electroencephalography (EEG). We use a 129-channel Geodesics (EGI, Oregon, USA) system designed for research purposes only. This EEG system utilises electrodes covered in sponges filled with a saline solution. Therefore there is no risk of skin or hair damage involved in this type of EEG recording. Participant's respiratory movements will be monitored using an ADInstruments (ADInstruments Ltd, Australia) respiratory monitor. The ADI system is a research purpose respiratory monitor which consists of a soft elasticated belt which the participant will wear around their abdomen. The monitor will offer information about timing of inspirations and expirations. Two ADI thermistor electrodes will also be attached to the olfactometer nosepiece and positioned approximately 2 cm from the participant's nostrils, to further confirm the onset of inspirations. An ADI galvanic skin response (GSR) monitor will be attached to the second and third fingers of the participant's left hand to monitor autonomic system changes in the form of skin conductance changes. None of this monitoring equipment should cause any pain or discomfort to the participant.

Thirty participants are required for the pilot experiment, and twenty (DIFFERENT) participants are required for the main experiment. All applicants will be made aware that they can withdraw at any point in the experiment if they feel uncomfortable. They will also be informed that they have the right to withdraw their data after the experiment has ended. Miss Stephanie Cook and/or Dr Andrej Stancak and/or Dr Nicholas Fallon and/or Miss Hazel Wright will be present to assist with experiments. Miss Stephanie Cook will be responsible for controlling the stimulus presentation and monitoring the participants.

B3) List any research assistants, sub-contractors or other staff not named above who will be involved in the research and detail their involvement.

B4) List below all research sites, and their Lead Investigators, to be included in this study.

Research Site	Individual Responsible	Position and contact details
Eleanor Rathbone Building, University of Liverpool	Dr Andrej Stancak	Senior lecturer (contact as above)

B5) Are the results of the study to be disseminated in the public domain?

YES *(PLEASE DELETE AS APPLICABLE)*

- ***If not, why not?***

B6) Give details of the funding of the research, including funding organisation(s), amount applied for or secured, duration, and UOL reference

Funding Body	Amount	Duration	UoL Reference
Unilever	£35,000	3 years	JXR 10826
ESRC	£56,000	3 years	

B7) Give details of any interests, commercial or otherwise, you or your co-applicants have in the funding body.

SECTION C - EXPEDITED REVIEW

C1)

a) Will the study involve recruitment of participants outside the UK? *For studies involving overseas sites, please ensure you have researched any local approvals that might be required. Wherever possible this should include local research ethics approval. In the absence of a research ethics approval body, other relevant local approvals should be obtained, e.g. authorisation from a site, letter from a local organisation or group etc.*	NO
b) Does the study involve participants who are particularly vulnerable or unable to give informed consent? *(e.g. children, people with learning or communication disabilities, people in custody, people engaged in illegal activities such as drug-taking, your own students in an educational capacity) (Note: this does not include secondary data authorised for release by the data collector for research purposes.)*	NO
c) Will the study require obtaining consent from a "research participant advocate" (for definition see guidance notes) in lieu of participants who are unable to give informed consent? *(e.g. for research involving children or, people with learning or communication disabilities)*	NO
d) Will it be necessary for participants, whose consent to participate in the study will be required, to take part without their knowledge at the time? *(e.g. covert observation using photography or video recording)*	NO
e) Does the study involve deliberately misleading the participants?	NO
f) Will the study require discussion of sensitive topics that may cause distress or embarrassment to the participant or potential risk of disclosure to the researcher of criminal activity or child protection issues? *(e.g. sexual activity, criminal activity)*	NO
g) Are drugs, placebos or other substances (e.g. food substances, vitamins) to be administered to the study participants or will the study involve invasive, intrusive or potentially harmful procedures of any kind?	NO
h) Will samples (e.g. blood, DNA, tissue) be obtained from participants?	NO
i) Is pain or more than mild discomfort likely to result from the study?	NO
j) Could the study induce psychological stress or anxiety or cause harm or negative consequences beyond the risks encountered in normal life?	NO
k) Will the study involve prolonged or repetitive testing?	NO
l) Will financial inducements (other than reasonable expenses and compensation for time) be offered to participants?	NO

C2)

a) Will the study seek written, informed consent?	YES
b) Will participants be informed that their participation is voluntary?	YES
c) Will participants be informed that they are free to withdraw at any time?	YES
d) Will participants be informed of aspects relevant to their continued participation in the study?	YES
e) Will participants' data remain confidential?	YES
f) Will participants be debriefed?	YES

If you have answered 'no' to all items in SECTION C1 and 'yes' to all questions in SECTION C2 the application will be processed through expedited review.

If you have answered "Yes" to one or more questions in Section C1, or "No" to one or more questions in Section C2, but wish to apply for expedited review, please make the case below. See research ethics website for an example "case for expedited review".

C3) **Case for Expedited Review –** *To be used if asking for expedited review despite answering YES to questions in C1 or NO to answers in C2.*

SECTION D - PARTICIPANT DETAILS

D1) How many participants will be recruited?

30 participants will be recruited for the pilot experiment, 20 for the main EEG experiment.

D2) How was the number of participants decided upon?

The number of participants was estimated based on previous EEG studies utilising ERP and ERD analysis to investigate a variety of sensory modalities (Cheyne et al. , 2003, Gaetz and Cheyne, 2006, Stancak, 2006) and olfaction itself (Klemm et al., 1992, Martin et al., 1995).

D3)

a) Describe how potential participants in the study will be identified, approached and recruited.

This study will use opportunity sampling. An advertisement poster will be placed on campus notice boards, in common areas and on the university website, whereby an office phone number and email address will be available for those interested in the study to gather further information. Interested parties who contact the experimenter will be sent an information sheet detailing the nature of the experiment and asked whether they would be interested in participation. If they agree to take part, a suitable time and date will be arranged for a screening session prior the experiment, whereby participants will be tested to ensure that they can discriminate a number of different odours and screened to confirm the inclusion criteria before arranging a suitable time for the experiment.

b) Inclusion criteria:

Healthy males and females between the ages of 18-35 who are able to accurately discriminate between different odours.

c) Exclusion criteria:

People who suffer from asthma or allergies to fragrances or fragranced products will be excluded for safety reasons as the experiment involves delivery of low intensity pleasant and unpleasant odours. Participants who report anosmia or hyposmia (lack of, or impaired, sense of smell) or those who score less than 9 on the initial odour discrimination screening will be excluded as they may be less likely to be affected by pleasant odours which could confound the resulting data. Participants reporting neurological disorders will be excluded as this may affect their EEG recording. Participants over the age of 35 will be excluded as this is the age when we begin to see a reduction in the number of olfactory receptors in the nose.

d) Are any specific groups to be excluded from this study? If so please list them and explain why:

N/A

e) Give details for cases and controls separately if appropriate:

N/A

f) Give details of any advertisements:

An advertisement outlining the details of the study and participant inclusion and exclusion criteria will be posted on suitable advertising boards around campus, and electronically on the University website.

D4)

a) State the numbers of participants from any of the following vulnerable groups and justify their inclusion

Children under 16 years of age:	0
Adults with learning disabilities:	0
Adults with dementia:	0
Prisoners:	0
Young Offenders:	0
Adults who are unable to consent for themselves:	0
Those who could be considered to have a particularly dependent relationship with the investigator, e.g. those in care homes, students of the PI or Co-applicants:	0
Other vulnerable groups (please list):	0

b) State the numbers of healthy volunteer participants:

Healthy Volunteers	**30 (pilot) + 20 (EEG experiment)**

D5)

a) Describe the arrangements for gaining informed consent from the research participants.

A written consent form will be provided and signed by the participant and the researcher prior to the start of the experiment. The participants will be given the opportunity to ask any questions they may have before and after the experiment. They will be made aware of their right to withdraw at any time. The experiment will be attended by the principle investigator (Stephanie Cook) and also by either of the other researchers (Nicholas Fallon, Hazel Wright) or the project co-ordinator (Dr A Stancak).

b) **If participants are to be recruited from any of the potentially vulnerable groups listed above, give details of extra steps taken to assure their protection, including arrangements to obtain consent from a legal, political or other appropriate representative in addition to the consent of the participant** *(e.g. HM Prison Service for research with young offenders, Head Teachers for research with children etc.).*

N/A

c) **If participants might not adequately understand verbal explanations or written information given in English, describe the arrangements for those participants** *(e.g. translation, use of interpreters etc.)*

N/A

d) **Where informed consent is not to be obtained (including the deception of participants) please explain why.**

N/A

D6) **What is the potential for benefit to research participants, if any?**

No direct benefit, but participants will gain an insight into the psychology of olfaction.

D7) **State any fees, reimbursements for time and inconvenience, or other forms of compensation that individual research participants may receive. Include direct payments, reimbursement of expenses or any other benefits of taking part in the research?**

Each participant will receive £15 as reasonable compensation for time and expenses incurred during participation in the experiment.

SECTION E - RISKS AND THEIR MANAGEMENT

***NOTE**: Completing section E fulfils the requirement for risk assessment, provided that this section is reviewed if circumstances change, or new information makes it necessary.*

A copy of this form should be given to your departmental safety coordinator to enable monitoring of risk assessments. The findings of the risk assessment, especially the precautions required, must be communicated in a user-friendly way to all those doing this work.

E1) Describe in detail the potential physical or psychological adverse effects, risks or hazards (minimal, moderate, high or severe) of involvement in the research for research participants.

The risks to participants associated with exposure to low intensity, pleasant and unpleasant odours delivered using an olfactometer are minimal. The odours used are of a very low intensity and are not capable of producing nausea or discomfort. EEG is a non-invasive procedure and the risks associated with this technique are also minimal with participants merely required to have an elasticated mesh of wet sponge electrodes applied to their scalp.

E2) Explain how the potential benefits of the research outweigh any risks to the participants.

Any psychological or health risks associated with this research are minimal. The effects of odours on ongoing brain activity and evaluations of neutral stimuli are poorly understood and therefore the findings will be of great scientific value.

E3) Describe in detail the potential adverse effects, risks or hazards (minimal, moderate, high or severe) arising from this research to the researchers or anyone else.

Risks to researchers associated with ambient low intensity odours in the laboratory are minimal. There is also a minimal risk (for example of trip hazards) associated with working in the EEG lab itself.

E4) What precautions will be in place to minimise the risks identified in E1 and E3?

Prior to experiments participants will be made aware that they can withdraw at any time. The study will use an EC approved olfactory stimulator (Dancer design UK Ltd) and the intensity of each odour stimulus will be low, producing a sensation whereby participants are merely able to recognise the presence of the odour. Should researchers or participants experience discomfort associated with the odour, experiments will be halted immediately. A Blueair Ltd air purifier is in place to clean the ambient environment of odour molecules between sessions or in the event of any discomfort. The EEG system requires the use of a saline solution conductant with a sponge based electrode system which minimises potential harm to participants compared to older systems which required abrasive action to the scalp. The saline solution used with this EEG system is of an ambient temperature to minimise participant discomfort. The risks associated with working in the EEG laboratory are carefully managed by adhering to health and safety requirements regarding trip hazards and safe set-up of computers and electronic

equipment. The labs layout is continually monitored by the departmental health and safety officer Nicola Williams.

E5) **Will individual or group interviews/questionnaires discuss any topics or issues that might be sensitive, embarrassing or upsetting, or is it possible that criminal or other disclosures requiring action could take place during the study** (e.g. during interviews/group discussions, or use of screening tests for drugs)**?**

NO *(PLEASE DELETE AS APPLICABLE)*

- ***If Yes, give details of procedures in place to deal with these issues.***

N/A

E6) **Describe the measures in place in the event of any unexpected outcomes or adverse events to participants arising from their involvement in the project**

The experiment will take place in the EEG lab of the School of Psychology Building, where the lab is specifically designed for such experimental studies. Project co-ordinator Dr Andrej Stancak is responsible for the lab with a certificate as the first aider, and is also informed about the safety measures to be adopted in case of fire, or sudden health problem of a participant. In the event of serious adverse events researchers will follow the University's procedure for reporting the event. It will be the responsibility of the principle investigator to halt the study and notify the university via email (ethics@liv.ac.uk) in the event of a serious adverse event.

E7) **Explain how the conduct of the project will be monitored to ensure that it conforms with the study plan and relevant University policies and guidance.**

The principle investigator (Miss Stephanie Cook) will closely monitor the entire project and receive guidance from the project co-ordinator Dr Andrej Stancak

SECTION F - DATA ACCESS AND STORAGE

F1) Where the research involves any of the following activities at any stage (including identification of potential research participants), state what measures have been put in place to ensure confidentiality of personal data *(e.g. encryption or other anonymisation procedures will be used).*

**PLEASE NOTE THAT UNLESS THERE ARE EXCEPTIONAL CIRCUMSTANCES, ALL DATA MUST BE HELD SECURELY ON THE "M" DRIVE AND IN LINE WITH UNIVERSITY POLICY. VISIT THE CSD WEBPAGES FOR FURTHER INFORMATION*

Electronic transfer of data by magnetic or optical media, e-mail or computer networks	Participants will be numbered, there will be only one copy of numbers and corresponding names that will be kept on one computer only and disposed of after the study has been conducted.
Sharing of data with other organisations	Unilever will receive results of the study in the form of a report containing statistical analyses of behavioural and EEG data. To protect participant confidentiality, all data in the report will be entirely encoded and anonymous with no personal information or details which could identify specific participants.
Export of data outside the European Union	NO
Use of personal addresses, postcodes, faxes, e-mails or telephone numbers	NO
Publication of direct quotations from respondents	NO
Publication of data that might allow identification of individuals	NO
Use of audio/visual recording devices	NO
Storage of personal data on any of the following:	
Manual files	In locked filing cabinets in Room 2.04b of the Eleanor Rathbone Building
Home or other personal computers	*NO*
University computers	DATA WILL ONLY BE STORED ON THE UNIVERSITY'S SECURE SEVER (M: DRIVE). This drive is secure and password protected.
Private company computers	*NO*
Laptop computers	*NO*

F2) Who will have control of and act as the PRIMARY custodian for the data generated by the study?

PRINCIPAL INVESTIGATOR

F3) Who will have access to the data generated by the study?

The principle investigator (Miss Stephanie Cook) and project co-ordinator (Dr Andrej Stancak).

F4) For how long will data from the study be stored?

Data will be stored for a period of five years as this is the period requested by science journals.

SECTION G – PEER REVIEW AND TRAINING

G1) a) Has the project undergone peer review?

NO *(PLEASE DELETE AS APPLICABLE)*

b) If yes, by whom was this carried out? *(please enclose evidence if available)*

N/A

G2) a) What date was your most recent training in research ethics?

Date:

b) Please provide details of the training provider and course:

Training provider:	
Course title:	

SECTION H - CHECKLIST OF ENCLOSURES

	PLEASE ADD "YES" WHERE APPROPRIATE
Study Plan / Protocol	YES
Recruitment advertisement	YES
Participant information sheet	YES
Participant Consent form	YES
Research Participant Advocate Consent form	YES
Evidence of external approvals	
Questionnaires on sensitive topics	
Interview schedule	
Debriefing material	YES
Other (please specify)	
Evidence of peer review (If G1 = Yes)	

Appendix 2: Sample odour safety data sheets

SAFETY DATA SHEET
according to Regulation (EC) No. 1907/2006

symrise
always inspiring more...

222569 JASMIN FLAVOUR

Version 3 | Revision Date 10.04.2013 | Print Date 16.05.2014

SECTION 1: IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

1.1 Product identifier

Commercial Product Name	:	JASMIN FLAVOUR
Material number	:	222569
EC-No.	:	200-338-0

1.2 Relevant identified uses of the substance or mixture and uses advised against

Use of the Substance/Mixture	:	Flavor mix

1.3 Details of the supplier of the safety data sheet

Company	:	Holzminden Muehlenfeldstrasse 1 D-37603 Holzminden
Telephone	:	+495531900
Telefax	:	+495531901649
E-mail address	:	sds@symrise.com

For further information, please contact:
Symrise AG - Tel.: +49 / (0)5531 / 90-0

1.4 Emergency telephone number

Symrise AG - Tel.: +49 / (0)5531 / 90-0

SECTION 2: HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

Classification (REGULATION (EC) No 1272/2008)

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

Classification (67/548/EEC, 1999/45/EC)

Not a hazardous substance or mixture according to EC-directives 67/548/EEC or 1999/45/EC.

2.2 Label elements

Labelling (REGULATION (EC) No 1272/2008)

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

Labelling (67/548/EEC, 1999/45/EC)

Symbol(s)	:	None.
R-phrase(s)	:	None.
S-phrase(s)	:	None.

SAFETY DATA SHEET
according to Regulation (EC) No. 1907/2006

symrise
always inspiring more...

268908 METHYL MERCAPTAN 1% PG

Version 7 | Revision Date 24.01.2014 | Print Date 16.05.2014

SECTION 1: IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

1.1 Product identifier

Commercial Product Name : METHYL MERCAPTAN 1% PG

Material number : 268908

1.2 Relevant identified uses of the substance or mixture and uses advised against

no data available

1.3 Details of the supplier of the safety data sheet

Company : Holzminden
Muehlenfeldstrasse 1
D-37603 Holzminden

Telephone : +495531900

Telefax : +495531901649

E-mail address : sds@symrise.com

For further information, please contact:
Symrise AG - Tel.: +49 / (0)5531 / 90-0

1.4 Emergency telephone number

Symrise AG - Tel.: +49 / (0)5531 / 90-0

SECTION 2: HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

Classification (REGULATION (EC) No 1272/2008)

Flammable liquids, Category 3	H226
Chronic aquatic toxicity, Category 3	H412

Classification (67/548/EEC, 1999/45/EC)

R10
R52/53

2.2 Label elements

Labelling (REGULATION (EC) No 1272/2008)

Hazard pictograms :

Signal word : Warning

Hazard statements	:	H226	Flammable liquid and vapour.
		H412	Harmful to aquatic life with long lasting effects.

Appendix 3: Sample participant consent form

UNIVERSITY OF LIVERPOOL

Committee on Research Ethics

PARTICIPANT CONSENT FORM

Title of Research Project: Brain responses to visual and olfactory stimuli

Researcher(s): Stephanie Cook, Andrej Stancak, Nicholas Fallon, John Tyson-Carr

	Please initial box
1. I confirm that I have read and have understood the information sheet dated February 2015 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my rights being affected. In addition, should I not wish to answer any particular question or questions, I am free to decline.	☐
3. I understand that, under the Data Protection Act, I can at any time ask for access to the information I provide and I can also request the destruction of that information if I wish.	☐
4. I agree to take part in the above study.	☐

Participant Name	Date	Signature
Name of Person taking consent	Date	Signature
Researcher	Date	Signature

Student Investigator:
Stephanie Cook
Eleanor Rathbone Building Room 2.04b
01517946956
s.cook2@liverpool.ac.uk

Project supervisor:
Dr Andrej Stancak
Eleanor Rathbone Building Room 2.04a
01517946951
stancak@liverpool.ac.uk

Appendix 4: Sample participant screening form

UNIVERSITY OF LIVERPOOL

Name: _____ Male/Female (delete as appropriate)

Handedness: Right/Left

Email: _____ Head Circumference:

Age: ___ N-I: LPA-RPA:

---

1. Aged 18-35 ☐
2. Non-asthmatic ☐
3. Score on Sniffing test (minimum of 9 required) ☐
4. No neurological disorders – olfactory/epilepsy ☐
5. No odour allergies ☐

Any other medication/medical conditions: _____

Approved for participation in experimental sessions ☐

Participant Code:_____

Initial Face Ratings complete ☐

	Pleasantness		Intensity		Familiarity	
	Before	After	Before	After	Before	After
Clean Air						
Jasmine						
Methylmercaptan						