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Human Olfactory Perception:
Mechanisms, Characteristics, and Functions

by

Jennifer Chen

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APPROVED, THESIS COMMITTEE:

Denise Chen, Associate Professor, Director
Neurology, Baylor College of Medicine

James Pomerantz, Professor, Chair
Psychology

Jessica Logan, Assistant Professor
Psychology

Casey O'Callaghan, Associate Professor
Philosophy

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ABSTRACT

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Olfactory sensing is ubiquitous across animals and important for survival. Yet, its characteristics, mechanisms, and functions in humans remain not well understood. In this dissertation, I present four studies on human olfactory perception. Study I investigates the impact of short-term exposures to an odorant on long-term olfactory learning and habituation, while Study II examines human ability to localize smells; Study III probes visual-olfactory integration of object representations, and Study IV explores the role of olfaction in sensing nutrients. Several conclusions are drawn from these studies. First, brief intermittent exposures to even a barely detectable odorant lead to long-term incremental odorant-specific habituation. Second, humans localize smells based on gradient cues between the nostrils. Third, there is a within-hemispheric advantage in the integration of visual-olfactory object representations. Fourth, olfaction partakes in nutrient-sensing and facilitates the detection of food. Some broader implications of our findings are discussed.
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BACKGROUND

This dissertation starts from a review of what we have known about the mechanisms, characteristics and functions of the human olfactory system, followed by questions that remain unclear and equivocal within this field, which lead to a series of studies in this dissertation.

Neuroanatomical Overview of Human Olfactory System

Humans are capable of detecting thousands of different smells with about 384 intact G-protein-coupled olfactory receptors (ORs) (Menashe, Aloni, & Lancet, 2006). The ORs are located in the cilia of the olfactory sensory neurons (OSNs) on each side of the upper nasal cavity, termed olfactory mucosa. Binding of the odorant molecule to the ORs sends the olfactory information via electrical signals in the OSNs to the ipsilateral olfactory bulb (OB) on each side of the hemisphere (Powell, Cowan, & Raisman, 1965). The OSNs, expressing the same OR type, converge stereotypically to a fixed number of glomeruli (about 2 glomeruli per OR in mice and rats and about 16 glomeruli per OR in humans) in each bulb where they synapse with the mitral and tufted cells (Maresh, Rodriguez Gil, Whitman, & Greer, 2008). Olfactory information continues ipsilaterally from olfactory bulb to the anterior olfactory nucleus (AON), the olfactory tubercle, the piriform cortex, the amygdala, and the entorhinal cortex (input regions from the OB that are collectively known as the primary olfactory cortex) and further on to the orbitofrontal cortex (OFC) (a major part of the secondary olfactory cortex) (Gottfried & Zald, 2005; Powell et al., 1965). Noticeably, olfaction is the only sensory system that has direct access to the OFC and amygdala, regions important for emotion processing, bypassing
the thalamus (Getchell & Shepherd, 1978a; Gottfried & Zald, 2005; Tanabe, Yarita, Lino, Ooshima, & Takagi, 1975).

In addition to the strong ipsilateral projection, AON sends a branch of olfactory information to the contralateral olfactory bulb via the anterior commissure (Brunjes, Illig, & Meyer, 2005; Lohman & Mentink, 1969), providing a route for the transfer of binaral inputs (R. B. Kay, Meyer, Illig, & Brunjes, 2011).

Finally, the olfactory bulbs not only feed forward the information to higher cortical levels, but also receive higher order modulation of the inhibitory interneurons called granule cells via centrifugal inputs from primary olfaction projection areas (Carmichael, Clugnet, & Price, 1994; Shepherd, 1998; Singer, Kim, & Zochowski, 2007).

**Characteristics of Human Olfaction**

Olfaction is one of the earliest emerging sensory systems in the fetus used to detect odorant molecules in the environment (Browne, 2008). While visual and auditory receptors are protected inside the sensory structures, olfactory receptors are barely protected and constantly exposed to chemicals, virus and dirt, which may be why olfactory receptors are renewed every 4-6 weeks throughout life (Graziadei, 1973). Different from other sensory pathways, olfactory inputs are projected to ipsilateral primary olfactory cortex (Powell et al., 1965; Price, 1973) via its own information flow control – olfactory bulb, instead of through thalamus (L. M. Kay & Sherman, 2007).

Greater individual variations are observed in olfaction than vision and audition (Köster, 2002). For example, olfactory thresholds to amyl acetate (a banana-like smell), pyridine (a spoiled milk-like smell), carvone (a minty smell) and cineole (a eucalyptus-like smell) vary by a factor of 1,000 across individuals (Koelega & Köster, 1974;
Lawless, Thomas, & Johnston, 1995). Androstenone (5α-androst-16-en-3-one), a steroid found in human sweat and urine, can be described as offensive (sweaty or urinous), pleasant (sweet or flowral) and odorless by different individuals (Bremner, Mainland, Khan, & Sobel, 2003; Keller, Zhuang, Chi, Vosshall, & Matsunami, 2007; Wysocki & Beauchamp, 1984).

In terms of the cognitive process of olfactory information, humans are notoriously bad at naming odors, but much better at discriminating odors based on olfactory qualities and pleasantness (Cain & Potts, 1996; Köster, 2002; Yeshurun & Sobel, 2010). In fact, olfaction is also characterized as a “hidden sense” (Köster, 2002). That is, olfactory information acts more strongly when it is processed out of conscious awareness; it subliminally guides human psychology and physiology, conjuring up greater effect on individuals than when the smells are explicitly processed (W. Li, Moallem, Paller, & Gottfried, 2007; Sela & Sobel, 2010).

As part of the sensory system, it is not surprising that olfaction shares certain sensory mechanisms with other sensations while simultaneously possessing its own unique properties. For instance, to respond to the dynamic environment and avoid overloading the brain with redundant information, sensory habituation mechanism is universally evolved to extract the changing inputs from the background and reduce responsiveness to the repeated and static stimulations (O’Mahony, 1986; Stevenson & Wilson, 2007). Nevertheless, the magnitude of perceived intensity drops to asymptote within 1 min in olfaction (Berglund, Berglund, & Lindvall, 1978) while it takes more than 3 min and 15 min in audition (Jerger, 1957) and vision (Redding, 1973), respectively. In another example, most animals have two eyes, two ears and two nostrils. One advantage of
having paired sensory organs is that the stimulus is perceived as more intense and clear (Blake & Fox, 1973; Doty, Soiffer, & Hummel, 1998; Hawley, Litovsky, & Culling, 2004). The other benefit is stereo perception, including depth perception and sound localization, by comparing intensive and temporal difference between the two sensory organs (Catania, 2013; Middlebrooks & Green, 1991; Parker, 2007; J. Porter et al., 2007). However, spatial localization ability in human olfaction is still equivocal. Termed lateralization, trigeminal, or localization task, subjects are asked to indicate the nostril that is presented with the smell while the other nostril is presented with diluent or clean air. Successful localization indicates that the smell is trigeminal; otherwise, the smell is a pure odorant (Boyle, Lundström, Knecht, Jones-Gotman, & Hummel, 2006; Wysocki, Cowart, & Radil, 2003). With a few exceptions (Porter, Anand, Johnson, Khan, & Sobel, 2005; von Bekesy, 1964), it is widely held that only trigeminal smells can be localized. Therefore, it remains uncertain whether the human olfaction system is equipped with similar spatial localization mechanism as in vision and audition.

**Function of the Olfactory System**

Olfaction is critical for survival and the well-being of many animals (Ache & Young, 2005). Animals rely on it to detect and localize food, predators, and home; find a mate; demarcate self and others, in-groups and out-groups, and dominance and subordinance; signal affective and motivational state; and warn fellow conspecifics of pending danger (Brennan & Zufall, 2006; Dulac & Torello, 2003; Wyatt, 2003). Moreover, olfaction is an integral part of flavor perception (Shepherd, 2006) and alerts people of danger from spoiled food, gas leak, and fire (T. Hummel & Nordin, 2005; Miwa et al., 2001). People with olfactory impairment often complain of lower quality of life; they enjoy food less,
worry more about their safety, and have more hygiene concerns, and a higher incidence of depression (Hummel & Nordin, 2005; Miwa et al., 2001).


Olfactory disorders often go unreported (Temmel et al., 2002) but can signal serious underlying neurological diseases. The amyloid-plague-forming protein that is associated with the loss of cognitive functions in the Alzheimer’s disease first build up in the olfactory brain regions (Kovács, 2004; Reyes, Deems, & Suarez, 1993). As a result, impaired olfaction often proceeds cognitive dysfunction and serves as an early indicator of the disorder (Kovács, 2004; Mesholam, Moberg, Mahr, & Doty, 1998). Plague built-up in the olfactory region is also believed to be linked to olfactory deficits in other neurological conditions that range from Parkinson’s disease to multiple sclerosis (Doty, Li, Mannon, & Yousem, 1998; Lutterotti et al., 2011).

The brain integrates signals from different senses to enhance the saliency of meaningful events and enlighten our perception of the world. Therefore, as compared to vision and audition, human olfaction alone may not be as essential as vision and audition,
yet its function is more obvious when olfaction orchestrates with other senses, including vision and gustation.

*Visual-olfactory integration*

As the saying goes, “eyes are the windows to the soul.” Vision is commonly accepted as the dominant sense over olfaction and other senses. Indeed, visual influences on olfaction have been well documented. However, recent evidence has shown that vision is influenced by olfaction when visual inputs are unstable and unreliable.

*Visual influence on olfaction*

The founding father of modern psychology, William James, wrote, “We know that a weak smell or taste may be very diversely interpreted by us, and that the same sensation will now be named as one thing and the next moment as another… In this wise one may make a person taste or smell what one will, if one only makes sure that he shall conceive of beforehand as we wish by saying to him ‘Doesn’t it smell just like, etc.?’” (James, 1890). Parallel with this idea, it has been widely reported that visual information such as colors, verbal labels, and pictures, often override the true nature of the olfactory percepts. Color, for one, has been consistently shown to influence odor identification, discrimination, intensity, and pleasantness judgments. Subjects were more likely to report detecting a smell when the substances were colored than without coloring (Engen, 1972). In an odor-color matching task, subjects matched certain odors to particular colors, such as bergamot-yellow, and cucumber-green (Demattè, Sanabria, & Spence, 2006; Gilbert, Martin, & Kemp, 1996; Schifferstein & Tanudjaja, 2004). When the solutions were colored inappropriately, such as cherry-orange, lemon-red, orange-yellow and white wine-red, more errors were made in odor identification task (Blackwell, 1995; DuBose,
Cardello, & Maller, 1980; Morrot, Brochet, & Dubourdieu, 2001; Zellner, Bartoli, & Eckard, 1991). Response accuracy in speeded odor discrimination between strawberry and lemon smells declined when incongruent color and shape cues (e.g., a lemon smell and a picture of a red lemon, a lemon smell and a picture of yellow strawberry) were presented (Demattè, Sanabria, & Spence, 2009). When lemon and grapefruit solutions were dyed red or green rather than yellow, the triangular discrimination test, in which subjects were asked to pick the odd odorant in inappropriate color-odor pairs, became more difficult (Stevenson & Oaten, 2008). Furthermore, the perceived intensity of the smell was arranged in sequence by the color saturation of the smell solutions (Belkin, Martin, Kemp, & Gilbert, 1997; Blackwell, 1995). When the odor solutions (e.g., strawberry smell) were colored (e.g., red), the smell was perceived more intense than colorless (Zellner & Kautz, 1990). In addition, subjects liked the odor with a compatible color (e.g., lemon-yellow) more than an incompatible one (e.g., lemon-red) (Zellner et al., 1991).

Verbal labels also affect olfactory perceptions. The presence of mismatched verbal labels impaired the performance on odor identification (Cain, 1979; Cain & Potts, 1996; Jehl, Royet, & Holley, 1997), as evidenced by the enhancement of P300 amplitudes in the context of rare or incorrect odor labels (Lorig, Mayer, Moore, & Warrenburg, 1993). Impertinent cues to the olfactory stimulus (e.g., a banana odor followed by a word ‘blue’) made the odor identification task more difficult (Davis, 1981). The odors with positive name (e.g., banana bread) were rated as more pleasant and less intense than odors with negative name (e.g., nail-polish remover) (Djordjevic et al., 2008). A mixture of isovaleric and butyric acids were labeled as “parmesan cheese” or “vomit”. Although the
olfactory stimuli remained the same, subjects rated the odor labeled “parmesan cheese” more pleasant than the same odor labeled “vomit” (Herz & von Clef, 2001). In the same vein, a test odor (isovaleric acid with cheddar cheese flavor) labeled “cheddar cheese” was judged more pleasant than the same test odor labeled “body odor”, in association with higher BOLD signal changes in rostral anterior cingulated cortex (ACC)/medial orbitofrontal cortex (OFC) and bilateral amygdala (de Araujo, Rolls, Velazco, Margot, & Cayeux, 2005). More strikingly, even in the absence of olfactory stimuli, odor-related words along, such as garlic, cinnamon, and jasmine, could elicit the activity in the olfactory regions, including bilateral piriform cortex and the right amygdale (González et al., 2006).

Pictures likewise influence olfactory perception. When an odor was presented with an appropriate picture (e.g., apple smell and a picture of an apple), subjects judged this odor more intense and pleasant than an odor presented with an inappropriate picture (e.g., apple smell and a picture of a pear) (Sakai, Imada, Saito, Kobayakawa, & Deguchi, 2005). In studies using event related potentials (ERP), perceiving mismatched odor-picture stimuli (e.g., grass smells and a picture of road surface) elicited significantly greater amplitude of the N400 peak which was thought to reflect the violation of expectancy, than perceiving matched odor-picture stimuli (e.g., grass smells and a picture of grass) (Grigor, Van Toller, Behan, & Richardson, 1999; Sarfarazi, Cave, Richardson, Behan, & Sedgwick, 1999).

Interestingly, pictures modulated olfactory perception more than the words in the binaral rivalry paradigm (J. Chen, Zhou, & Chen, 2012), in which subjects’ two nostrils were respectively presented with two different smells, inducing alternations between the
two olfactory percepts (Zhou & Chen, 2009c). Nevertheless, discrepant modulation effect between pictures and words did not appear in single smell presentation (J. Chen et al., 2012). Although verbal labels (words) and pictures both evoke perceptual and semantic object representations (Nelson, Reed, & McEvoy, 1977; Vandenberghe, Price, Wise, Josephs, & Frackowiak, 1996), pictures and words are distinguishable at the level of cognitive (Nelson et al., 1977; Theios & Amrhein, 1989) and neural representations (Grady, McIntosh, Rajah, & Craik, 1998; Menard, Kosslyn, Thompson, Alpert, & Rauch, 1996; Sevostianov et al., 2002; Vandenberghe et al., 1996). Therefore, visual modulation on olfaction is not solely driven by top-down, but also stimulus-driven processes, depending on the manner of smell presentations.

**Olfactory influence on vision**

While vision’s influence on olfaction has been well documented, not much is known about olfactory’s influence on vision. Due to the olfactory properties (e.g., hedonic values) alone or in combination with non-olfactory properties (e.g., trigeminal component), smell can elevate arousal level and draw more attention which, in turn, influences visual processing (Knasko, 1995; Michael, Jacquot, Millot, & Brand, 2005; Millot, Brand, & Morand, 2002). For example, in the presence of an odorant (e.g., an orange smell), subjects attended to the congruent visual object (e.g., a picture of oranges) for longer period of time, compared with the incongruent visual object (e.g., a picture of apples) (Seo, Roidl, Müller, & Negoias, 2010). On the higher cognitive level, the presence of a rose-like odor during semantic encoding of words (i.e., judging whether the words were animate or inanimate) resulted in poorer word recognition performance, suggesting the competition for semantic resource between olfaction and language (Walla, Hufnapl,
Lehrner, Mayer, Lindinger, Imhof, Deecke, & Lang, 2003a). Similar results were also found in face recognition tasks in which subjects’ face recognition performances declined when faces were encoded (i.e., by judging the sympathy of the faces) in the presence of an odor (Walla, Hufnagl, Lehrner, Mayer, Lindinger, Imhof, Deecke, & Lang, 2003b). Male faces were perceived less attractive in the presence of an unpleasant odor (e.g., rubber, body odor) than either a pleasant odor (e.g., geranium, a male fragrance) or clean air (Demattè, Osterbauer, & Spence, 2007).

Interestingly, olfaction exerts its influence on vision even when subjects are not aware of the presence of the olfactory cues. For example, subconscious positive (e.g., a rose smell) and negative (e.g., a rotten egg smell) smells facilitated the face recognition performance (Walla, Mayer, Deecke, & Lang, 2005). Moreover, smelling the sweat collected from donors undergoing fearful scenario modulated subject’s perception of facial emotions; being unaware of the identity and emotional valence of the smell, subjects perceived ambiguous facial expressions as more fearful in the presence of fearful sweat compared with pads that did not carry sweat (Zhou & Chen, 2009). In fact, one study showed that only in the absence of conscious awareness could the valence of olfactory cues bias subject’s preference for the neutral faces (Li, Moallem, Paller, & Gottfried, 2007).

In the latest study on olfactory influence on vision, Zhou and colleagues subjected subjects to a binocular rivalry paradigm where visual stimuli were unstable while subjects inhaled an odor that was congruent to one of the competing visual images (e.g., a rose smell and a rose image) (Zhou, Jiang, He, & Chen, 2010). They observed that odors
prolonged the perceived dominance of and shortened the suppression of congruent images, so olfactory influence on binocular rivalry is automatic and preconscious.

**Neural substrates of visual-olfactory integration**

Neuroanatomical pathways that connect the retinal and olfactory bulbs have been reported in primates and other mammals; they include the piriform cortex, the olfactory tubercle, the cortical region of the medial amygdale, lateral hypothalamus, and the bed nucleus of the stria terminals (Cooper, Mick, & Magnin, 1989; Cooper, Parvopassu, Herbin, & Magnin, 1994; Levine, Weiss, Rosenwasser, & Miselis, 1991; Mick, Cooper, & Magnin, 1993; Pickard & Silverman, 1981; Youngstrom, Weiss, & Nunez, 1991).

Odor objects are encoded as spatially distributed patterns in piriform cortex (Gottfried & Wu, 2009; Gottfried et al., 2006; Howard, Plailly, Grueschow, Haynes, & Gottfried, 2009; Illig & Haberly, 2003; Kadohisa & Wilson, 2006; Sharp, Kauer, & Shepherd, 1977; Wilson, 1997). Moreover, convergent visual and olfactory inputs elicit greater neural activities in the piriform cortex as compared with visual or olfactory input alone (Carmichael & Price, 1995; Cooper et al., 1994; Gottfried & Dolan, 2003; Pickard & Silverman, 1981; Youngstrom et al., 1991).

The OFC is known for receiving multisensory inputs, including vision, audition, olfaction, gestation and somatosensation (Carmichael & Price, 1995; Kringelbach, 2005; Rolls, 2004; Rolls & Baylis, 1994). Additionally, primate studies have found that the orbitofrontal cortex (OFC) receives and integrates a range of highly processed sensory inputs from primary olfactory cortex and ventral visual pathway (J. Price & Ongür, 2000; Rolls & Baylis, 1994). Neural activity in the OFC during visual-olfactory integration has also been well studied in human fMRI studies (Carmichael & Price, 1995; Cooper et al.,
1994; Gottfried & Dolan, 2003; Kringelbach, 2005; Osterbauer et al., 2005; Pickard & Silverman, 1981; Rolls, 2004; Rolls & Baylis, 1994; Youngstrom et al., 1991). For example, perceiving compatible color-odor pairs increased activity in the caudal OFC and the insular cortex (Osterbauer et al., 2005). Congruent smell and picture, such as a bus image and the diesel smell, enhanced the neural activity in the rostromedial OFC and the anterior hippocampus (Gottfried & Dolan, 2003).

**Olfactory-gustatory integration**

Juliet said, “A rose by any other name would smell as sweet.” Words used to describe taste are also commonly applied to smell, indicating the close connection between smell and taste (Burdach, Kroeze, & Köster, 1984; Prescott, 1999, 2010).

Smells are perceived in the nose via active sniffing (orthonasally) and in the mouth via chewing and swallowing food and beverages (retronasally) (Prescott, 2010; Rozin, 1982). The latter contributes significantly to the perception of flavor (Shepherd, 2006). With either route, volatile molecules stimulate olfactory epithelium, and reach the olfactory cortices via the olfactory bulbs (Prescott, 2010; Shepherd, 2006). As Rozin commented, “I argue that olfaction is the only dual sensory modality, in that it senses both objects in the external world and objects in the body (mouth). I suggest that the same olfactory stimulation may be perceived and evaluated in two qualitatively different ways, depending on whether it is referred to the mouth or the external world” (Rozin, 1982). In line with Rozin’s argument, the following reviews focus on how two olfactory routes interact differently with gustation.
**Retronasal olfactory-gustatory integration**

Flavor is an overall impression across multiple sensory systems, including olfaction, gustation, somatosensation, vision and audition, among which retronasal olfaction plays a significant role (Lawless, 2001; Shepherd, 2006). The classic demonstration can be dated back to 1880s – “Blindfold a person and make him clasp his nose tightly, then put into his mouth successively small pieces of beef, mutton, veal, and pork, and it is safe to predict that he will not be able to tell one morsel from another. The same results will be obtained with chicken, turkey, and duck; with pieces of almond, walnut, and hazelnut…” (Finck, 1886). In other words, identifying the food or beverages in the mouth when the nostrils are pinched shut is difficult (Frank & Byran, 1988). Moreover, the difference in preference for vegetables between people who liked and disliked vegetables was smaller when they wore nose clips (Lim & Padmanabhan, 2013). Furthermore, adding tasteless odors (e.g., strawberry or sardine smells) to perceptually congruent taste solutions (e.g., sucrose or sodium chloride) enhanced taste hedonic and intensity ratings (Cerf-Ducastel & Murphy, 2004; Schifferstein & Verlegh, 1996; Seo et al., 2011; de Araujo, Rolls, Kringelbach, McGlone, & Phillips, 2003).

In addition to the odor-induced taste enhancement, taste (e.g., sweet taste) facilitates retronasal olfactory processing of a perceptually congruent smell (e.g., vanilla smell) in association with shorter P2 latencies of olfactory event-related potentials (Welge-Lüssen, Husner, Wolfensberger, & Hummel, 2009).

**Orthonasal olfactory-gustatory integration**

Smell and taste integration is not limited to retronasal, but also orthonasal smell. When smells were presented through the nose and congruent tastes on the tongue (e.g.,
sweet taste-vanilla smell and salty taste-soy sauce smell), perceived intensity of smells and tastes was mutually enhanced by each other (Djordjevic, Zatorre, & Jones-Gotman, 2004; Green, Nachtigal, Hammond, & Lim, 2012; Sakai, Kobayakawa, Gotow, Saito, & Imada, 2001). Subjects also identified the tastes (e.g., sweet taste) faster in the presence of perceptual congruent (e.g., a strawberry smell) than incongruent smell (e.g., a grapefruit smell) (Djordjevic, 2004; White & Prescott, 2007). Even a subthreshold concentration of a sweet-tasting solution (saccharin) increased olfactory sensitivity to benzaldehyde (i.e., a cheery- and almond-like smell) (Dalton, Doolittle, Nagata, & Breslin, 2000).

**Neural substrates of olfactory-gustatory integration**

Neuroimaging research in humans have identified several overlapping brain areas, including insula, OFC, and ACC, that are activated during unimodal orthonasal, retronasal, and gustatory stimulations (Cerf-Ducastel & Murphy, 2001; de Araujo, Rolls, Kringelbach, McGlone, & Phillips, 2003; O’Doherty, Rolls, Francis, Bowtell, & McGlone, 2001; Poellinger et al., 2001; Savic, Gulyas, Larsson, & Roland, 2000; Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001).

In the absence of gustatory stimulations, orthonasal olfactory sensitivity to both food (e.g., a chocolate smell) and nonfood (e.g., a lavender smell) smells was higher than retronasal olfactory sensitivity, correlated with greater amplitudes and shorter latencies of olfactory event-related potentials (Heilmann & Hummel, 2004). On the other hand, in the presence of tastes (e.g., a sweet taste), orthonasal olfactory sensing congruent food smells (e.g., chocolate, strawberry smells) led to deactivations in insula, OFC, and ACC (Small, Jones-Gotman, Zatorre, Petrides, & Evans, 1997) while retronasal olfactory sensing
congruent food smells led to activations in the same areas (Seo et al., 2011; Small et al., 2004; de Araujo et al., 2003). Note that such dissociated neural responses of orthonasal and retronasal olfactory-gustatory integration was not observed in the nonfood smells, but food smells (Small, Gerber, Mak, & Hummel, 2005), suggesting that food related stimuli are represented differently in retronasal-gustatory and orthonasal-gustatory interactions (Small et al., 2005).

**Aims of Current Dissertation**

Behavioral and neural studies on human olfactory characteristics, mechanisms, and functions notwithstanding, equivocal issues remain. Firstly, habituation refers to a reduced sensitivity to a stimulus as a result of repetitive and continuous exposure. It is the simplest form of non-associative learning, constituting simple and complex behaviors, such as discriminating the target against the background accurately and attending to the important biological events effectively. In terms of the duration of habituation, olfactory habituation is commonly classified into short-term (minutes to hours) versus long-term (days to weeks) habituation. While much is known about short-term habituation, little is known about long-term habituation or the effect of short-term habituation over the long run.

Secondly, a fundamental function of olfaction is odor localization. The three dimensional world is encoded through pairs of human sensory organs. Each in a pair receives slightly different input from the other. For sight and sound, such gradient is well documented to enable spatial localization. Findings regarding human olfaction have largely been negative with mononaral odorant presentation to one nostril and clean air or diluent to the other nostril. Yet, such experimental paradigm neglects the gradients
between the nostrils. Can gradients between the nostrils enable olfactory localization in humans as they do in sight and sound? Do humans experience stereo olfaction as do other animals?

Thirdly, visual-olfactory interaction has been vistied and revisited in a wide range of behavioral and neural studies. While it is known that each individual sensory input is processed at different levels along sensory hierarchy and would interact with each other at some points, it is unclear at what cognitive stage the two sensations converge. In addition, does it occur at the level of semantic processing or sensory representation?

Last but not least, olfaction and taste collectively form flavor perception and guide food ingestive behaviors. While much is studied about the involvement of taste in sensing nutrients, little is known about the role of olfaction in this process. Do human noses sense the tastants? If so, does it facilitate us to search nutrient-rich food?

These will be addressed in four studies presented below. Study 3 on visual modulation of olfaction is done in collaboration with Wen Zhou, Xiaomeng Zhang, and Li Wang of the Chinese Academy of Sciences and published in the 2013 issue of the Journal of Neuroscience.
Introduction

Our sensory world is replete with information and sorting out the relevant from the irrelevant is critical for survival. Habituation serves this purpose by attenuating the response after repeated or prolonged exposure to the same stimulus, freeing up attention resources for novel stimuli. Depending on the stimulus intensity, exposure duration and frequency, habituation can be short-term, which has a span of minutes to hours, or long-term, which has a span of days to weeks (Dalton & Wysocki, 1996; Dalton, 2000; Maschke et al., 2000; Menard et al., 1996; Rankin et al., 2009). The characteristics of habituation have been well described in the classic review by Thompson and Spencer (1966) and the revised update (Rankin et al., 2009). Animal studies have further showed that short-term and long-term habituations involve metabotropic glutamate (mGluR) II/III receptor-mediated process in olfactory cortex and N-methyl-D-aspartate (NMDA) receptor-dependent process in olfactory bulb, respectively (Chaudhury et al., 2010; Wilson & Linster, 2008). Much of which, however, is based on work on anesthetized animals. Moreover, despite arguments on different cellular and molecular mechanisms underlying the short- and long-term habituation, behaviorally, the distinction between the two appears arbitrary (Harris, 1943). Do repeated short-term exposures to an odorant have any effect over the long run? If so, is it odorant-specific? And, is it simply a series of short-term habituations?
Understanding long-term olfactory habituation is important in its own right, as it more closely resembles real-world situations where people live and work in similar olfactory environment. Here we address these using the olfactory habituation paradigm, and examining the effect of short-term olfactory exposures on the subject over the long run.

Materials and Methods

Participants

10 healthy non-smoking women (mean age = 21.70 years, SEM = 1.63) with normal sense of smell (threshold of PEA in propylene glycol < 0.0625%) consented to participate in two separate experiments, with 5 women in each experiment. None reported any olfactory or upper respiratory infection at the time of the study.

Olfactory stimuli

Phenylethyl alcohol (PEA; a rose-like smell) and n-butanol (butanol; a sharpie marker-like smell) were used as the test and the control odorants, respectively in one experiment. The test and control odorants were reversed in the other experiment. Thresholds to the two odorants were assessed using Sniffin’ Sticks (Burghart Instruments, Weldel, Germany) in a triple-forced-choice ascending staircase with reversal design (Hummel, Sekinger, Wolf, Pauli, & Kobal, 1997; Kobal et al., 2000). Propylene glycol (PG) served as the blank in the threshold test.

Procedure

Subjects were tested individually in a temperature- and ventilation-controlled room. They were intermittently presented in each session with a barely detectable odorant using
an olfactory threshold paradigm. Specifically, a subject was presented with 3 Sniffin’ Sticks consisting of two blanks and one peri-threshold target PEA odorant, and asked to identify the one that contained the target. If the subject discerned the target correctly in two consecutive trials, a lower concentration was presented in the subsequent trial. Conversely, if the subject provided an incorrect response, a higher concentration was presented next. No feedback was provided. Intertrial interval was 30 sec to prevent sensory fatigue. The final threshold was determined by averaging the last four staircase reversal points among a total of seven reversals. During each session, subjects were exposed to the test odorant intermittently for a total of 35.10 sec (SEM = 0.63) (i.e., 2 sec per trial every 30 sec over an average of 17.55 trials). The threshold of the test odorant (PEA or butanol) was assessed every 3.82 days (SEM = 0.19) for each subject across two consecutive menstrual cycles for an average of 17.70 sessions (SEM = 0.63). Each subsequent session started at a concentration one dilution step below the threshold acquired from the subject’s previous session. The threshold of the control odorant (butanol or PEA) was assessed twice: once at the beginning and once at the end of the entire experiment.

Each individual participated over the course of two consecutive menstrual cycles. Because menstrual cycle phase, basal body temperature, and heart rate may each affect olfactory sensitivities (Doty, Snyder, Huggins, & Lowry, 1981; Navarrete-Palacios, 2003), subjects reported proactively the first and last day of their menses, recorded daily basal body temperature upon wakening in the morning, prior to any physical activities, and measured their heart rates prior to threshold assessments during each test session.
Data analyses

The effect of long-term repeated testing on olfactory sensitivity was assessed using a linear mixed model, with thresholds of the test odorant as the dependent variable, two key signatures of the menstrual cycle phase – ovulatory phase (yes vs. no) and menses (yes vs. no) – as the factors, basal body temperature and heart rate as the covariates, and test session (1 to up to 20) as the repeated variable. Ovulatory phase was defined as day 12 to 16 from the first day of the menstrual cycle (i.e., Day 1 of menses). The menstrual cycles of each subject were standardized to 28 days using \[\left(\frac{\text{the day in the menstrual cycle relative to Day 1}}{\text{the length of the menstrual cycle}}\right) \times 28\]. Paired t-tests were performed to compare the thresholds of control odorants before and after long-term repeated exposures to the test odorants.

Results and Discussion

Short-term intermittent exposures to peri-threshold PEA lead to long-term habituation to PEA

We found that subjects’ sensitivity to PEA declined significantly as a function of the number of sessions in which they were exposed to peri-threshold PEA \[F(1,75.03) = 23.72, P < 0.001; \text{Fig. 1}\]. Notably, the decline is monotonic, cumulative over time, and observable in all 5 subjects, despite large individual variations in their susceptibility to the habituation [correlation coefficients (r) ranging from 0.06 to 0.80].

We showed that this reduced sensitivity to PEA is due to repeated testing, as subjects’ sensitivity to an odorant that was not tested repeatedly remained unchanged \[t(4) = 1.92, P = 0.13; \text{Fig. 1}\].
Fig. 1. Repetitive intermittent exposures to peri-threshold PEA lead to long-term habituation to PEA. Mean odorant thresholds (in log millimolar concentration) across test sessions where PEA and butanol served as the test and control odorant, respectively. PEA thresholds increased as a function of the number of times subjects had been tested while thresholds of the control odorant butanol, assessed at the beginning and the end of the experiment, remained unchanged. Error bars represent standard errors of means.

Habituation to PEA lasts beyond the testing phase

One of the key characteristics of sensory habituation is that sensory response recovers spontaneously to baseline after target stimulus is withdrawn. Habituation to PEA continued throughout the experiment and we asked how long the effect would last beyond the experiment. We followed two of our original subjects (neither had contact with the PEA in the interim) for one and four sessions, respectively, 1.5 yrs after the experiment, testing their threshold to the target odorant PEA and the control odorant butanol in the same fashion as in the main experiment earlier. While the PEA threshold of one subject had recovered to its initial level, the PEA threshold of the second subject had only partially recovered (37% of the initial sensitivity). Notably, upon retesting, the second subject’s PEA threshold quickly returned to its habituated level at the end of the experiment 1.5 yrs ago, and remained so during subsequent testings (Fig. 2). However,
their threshold to the control odorant butanol remained unchanged from the original experiment.

![Graph of log threshold concentration (mM) vs. test session](image)

**Fig. 2. Habituation to PEA lasts beyond the testing phase.** PEA thresholds of one subject during the original experiment (sessions 1-20) and during the followup visits 1.5 years later (sessions 21-24). Her PEA thresholds increased after intermittent PEA threshold testing during the original experiment. During the followup experiment, her threshold was only partially recovered; notably, after just one re-testing, her threshold returned to the level at the end of the original experiment 1.5 years ago.

*Effect of long-term habituation from short-term intermittent exposures is odorant-specific*

We asked whether the effect we observed held across all odorants. We tested this by switching the target and control odorants and exposing a different matched group of female subjects to a peri-threshold target butanol and control PEA odorants in the same fashion as in the first experiment. Unlike it was for the PEA, short-term intermittent exposures to a peri-threshold butanol did not lead to long-term reductions in olfactory sensitivity to butanol \([F(1,72.31) = 3.18, P = 0.08; \text{Fig. 3}].\) Butanol is an unpleasant odorant that stimulates the trigeminal system (Doty et al., 1978) and triggers somatosensory sensitization (Hempel-Jørgensen, Kjærgaard, Mølhave, & Hudnell, 1999)
at higher concentrations, which may have contributed to the lack of long-term habituation effect at the peri-threshold concentration.

**Fig. 3. Repetitive intermittent exposures to a peri-threshold odor leads to long-term habituation – an odorant-specific effect.** Mean odorant thresholds (in log millimolar concentration) across test sessions where butanol and PEA served as the test and control odorant, respectively. Butanol thresholds remained unchanged across the sessions. Thresholds of the control odorant PEA, assessed at the beginning and the end of the experiment, remained the same. Error bars represent standard errors of means.

Our findings may appear at odd with the literature which shows that repeated threshold testings tend to enhance rather than reduce olfactory sensitivity (Cain & Gent, 1991; Dalton, Doolittle, & Breslin, 2002). Differences in smell presentation (Sniffin’ Sticks in our present study vs. bottles in other studies), odorant property (PEA in our study vs. benzaldehyde), number of different smells tested in the same session (one in our study vs. several in Cain), and inter-stimulus interval (4 days in our study vs. 1-2 days in others) (Thompson & Spencer, 1966), may contribute to the different response patterns observed here. Our findings, however, are consistent with both the original and more recent habituation literature showing the seemingly paradoxical phenomenon of greater habituation to weak or even subliminal stimuli (Harris, 1943; Kunst-Wilson & Zajonc,
Moreover, they are remarkably consistent with a recent two-photon calcium imaging study in awake mice which shows that brief (4s a day) and repeated (over 2 days) exposures across a wide range of odorants lead to gradual long-lasting experience-dependent reductions in mitral cell and enhanced activities in inhibitory granual cells (Kato, Chu, Isaacson, & Komiyama, 2012).

The long-term habituation that we observed likely results from the cumulative effect of learning and memory. Responses in the olfactory bulb, the first central processing station of olfactory information, can be modulated by centrifugal inputs from the cortex (e.g., piriform cortex, entorhinal cortex and amygdala) in association with olfactory learning and memory (Fletcher & Chen, 2010; Z. Li & Hertz, 2000; Potter & Chorover, 1976; Wilson & Sullivan, 1992). Prolonged exposure to an odorant can modify the spatiotemporal responses of mitral and tufted cells, the output neurons of the olfactory bulb (Eckert & Schmidt, 1985). Olfactory experience also shapes the physiological and anatomical organization in the piriform cortex (Brosh & Barkai, 2009; Fletcher & Chen, 2010; Saar, Grossman, & Barkai, 2002), orbitofrontal cortex (W. Li et al., 2006), and hippocampus (Deshmukh & Bhalla, 2003; Eichenbaum, Mathews, & Cohen, 1989; Gourévitch, Kay, & Martin, 2010; Poellinger et al., 2001). In particular, subgroups of hippocampal neurons encode the temporal dynamics of repeated odor stimuli (Deshmukh & Bhalla, 2003) and contextual traces associated with the odors (Redish, 2001). Such mechanism is especially important for the olfactory system that requires the organism to be constantly monitoring the fluctuations in target and background odors (Kadohisa & Wilson, 2006; Linster, Menon, Singh, & Wilson, 2009; Stevenson & Wilson, 2007).
To summarize, we show that brief and repeated intermittent exposures to an odorant can lead to long-term habituation to the odorant. This effect accumulates monotonically and is observed in all subjects despite individual variations in the magnitude of habituation and recovery rate. Notably, the long-term effect is enduring in some individuals, lasting well beyond the stimulus presentation, which is reminiscent of Thorpe (1956)’s description of habituation as a “permanent waning of a response as a result of repeated stimulation”. This finding is even more remarkable in light of the fact that the long-term effect was in response to a seemingly insignificant and barely detectable smell. Our findings challenge the conventional distinction between short- vs. long-term habituation (Thompson & Spencer, 1966; Thompson, 2009; Rankin et al., 2009). Moreover, they may shed new light on understanding incidental memory and the long-lasting nature of olfactory memory.

Conclusion

We report that short-term intermittent exposures to a peri-threshold odorant lead to long-term olfactory habituation. This effect accumulates monotonically and is observed in all subjects despite individual variations in the magnitude of habituation and recovery rate. Our findings defy conventional characterization of short-term and long-term habituations, and shed new light on understanding the basic mechanism of olfactory perception and memory in the simplest form of learning.
STUDY II: GRADED OLFATORY CONTRASTS BETWEEN NASAL PASSAGES ENABLE STEREO HUMAN OLFACTION

Introduction

Localizing objects in space is critical for survival. In animals with bilaterally symmetric sensory organs, subtle disparities between outputs to both sensory organs provide an important source of cues for spatial localization. For example, differences between viewpoints of the two eyes enable stereoscopic depth perception (Parker, 2007). Onset and loudness disparities between the sound waves reaching the two ears enable the localization of sound (Middlebrooks & Green, 1991). Likewise, gradients in concentration and arrival time between the two nostrils/antennae facilitate odor localization in honey-bee (Martin, 1965), bacteria (Macnab & Koshland, 1972), catfish (Johnsen & Teeter, 1980), lobsters (Atema, 1996; Gomez & Atema, 1996; Gomez-Marin, Duistermars, Frye, & Louis, 2010; Kozlowski et al., 1998; Moore, Scholz, & Atema, 1991), fruit flies (Borst & Heisenberg, 1982; Duistermars, Chow, & Frye, 2009; Gaudry, Hong, Kain, de Bivort, & Wilson, 2013; Louis, Huber, Benton, Sakmar, & Vosshall, 2008; Raman, Ito, & Stopfer, 2008), ants (Steck, Knaden, & Hansson, 2010), sharks (Gardiner & Atema, 2010), rats (Kikuta et al., 2010; Rajan, Clement, & Bhalla, 2006; Wilson & Sullivan, 1999) and moles (Catania, 2013).

However, evidence of human localization of olfaction is largely negative. While humans can track scents with head motion (J. Porter et al., 2007; Schneider & Schmidt, 1967; von Bekesy, 1964), it is widely held that humans cannot localize a pure odorant when the head is stationary (Frasnelli, Charbonneau, Collignon, & Lepore, 2009;
The typical localization paradigm involves mononaral odorant presentation where a single smell is presented in one nostril and a diluent, clean air, or occasionally, the same smell of a different concentration (Schneider & Schmidt, 1967), is presented in the other nostril. This paradigm is better known as the olfactory trigeminal test, with the assumption that only smells (e.g., amyl acetate and eucalyptol) that activate the trigeminal nerve can be successfully localized (Frasnelli et al., 2009, 2010; Kleemann et al., 2009; Kobal et al., 1989; Radil & Wysocki, 1998; Schneider & Schmidt, 1967; Wysocki et al., 2003). Few human olfactory localization studies, however, employ gradient cues between olfactory inputs to the nostrils in their design. Can gradients between the nostrils enable olfactory localization in humans as they do in sight and sound? Do humans experience stereo olfaction as do other animals?

We addressed this in 12 subjects by simultaneously presenting a pair of olfactory stimuli one to each nostril and varying the intensity (odorless, detectable, clearly detectable), identifiability (not identifiable, just identifiable, clearly identifiable), and quality (phenylethyl alcohol or PEA, a rose-like smell vs. eugenol or EUG, a clove-like smell) contrasts between the nostrils. Subjects sampled each pair of smells over the course of 40 trials and button pressed to indicate whether the target smell came from the left or right nostril.

**Materials and Methods**
Participants

12 right-handed healthy non-smokers with normal sense of smell (1 male, 11 females; mean age = 24.50, SEM = 1.89) consented to participate in the experiment. None reported respiratory allergy or upper respiratory infection for the duration of testing.

Olfactory stimuli

Phenylethyl alcohol (PEA, a rose-like smell) and eugenol (EUG, a clove-like smell) were chosen because they are pure odorants (Doty et al., 1978) that are not localized when the other nostril is presented with diluent or air (Frasnelli et al., 2010; Kobal et al., 1989; Schneider & Schmidt, 1967). PEA and EUG were diluted in propylene glycol (PG) from 4% v/v to form 30 and 50 binary dilution steps, respectively. PG was used as the blank. Each stimulus consisted of 10 ml of solution in a 280 ml glass bottle that was fitted with a Teflon nosepiece.

Procedure

Prior to the localization tasks, we measured each subject’s nostril-specific olfactory thresholds for PEA and EUG using triple-forced-choice ascending staircase with reversal design (Hummel et al., 1997; Kobal et al., 2000). The nostril that was not tested was blocked by a piece of surgical tape (Doty & Kerr, 2005). On each trial, subjects were presented with one target smell (PEA or EUG) and two blanks and asked to identify the target smell. An incorrect response and two consecutive correct responses would respectively lead to a higher and a lower testing concentration in the next trial. A reversal occurred when the concentration series changed direction. Thresholds were determined by taking the average of the last four reversals. Mean thresholds were $2.02 \times 10^{-5}$ M (SEM = $7.24 \times 10^{-6}$) and $1.98 \times 10^{-5}$ M (SEM = $8.05 \times 10^{-6}$), respectively in the left and
the right nostrils for the PEA, and $7.54 \times 10^{-6}$ (SEM = $4.23 \times 10^{-6}$) and $4.40 \times 10^{-6}$ (SEM = $3.68 \times 10^{-6}$), respectively in left and right nostrils for the EUG. Tailored to each subject’s nostril-specific olfactory sensitivity, 3 concentrations of PEA and EUG each were used in subsequent localization tasks and referred to here as “odorless and unidentifiable” (-; approximately 5 dilution steps below the thresholds, equivalent to $1.13 \times 10^{-7}$ M PEA- in the left nostril, $2.08 \times 10^{-7}$ M PEA- in the right nostril, $2.24 \times 10^{-8}$ M EUG- in the left nostril and $2.70 \times 10^{-8}$ M EUG- in the right nostril), “detectable and just identifiable” (+; approximately 5 dilution steps above the PEA thresholds and 10 dilution steps above the EUG thresholds, equivalent to $8.40 \times 10^{-4}$ M PEA+ in the left nostril, $8.33 \times 10^{-4}$ M PEA+ in the right nostril, $2.27 \times 10^{-4}$ M EUG+ in the left nostril and $5.23 \times 10^{-4}$ M EUG+ in the right nostril), to “clearly detectable and identifiable” (++; approximately 10 dilution steps above the PEA thresholds and 15 dilution steps above the EUG thresholds, equivalent to $1.98 \times 10^{-2}$ M PEA++ in the left nostril, $1.69 \times 10^{-2}$ M PEA++ in the right nostril, $3.60 \times 10^{-3}$ M EUG++ in the left nostril and $5.65 \times 10^{-3}$ M EUG++ in the right nostril).

Subjects were given the opportunity to sample the target smell at the beginning of each localization task. All identified the suprathreshold target smells correctly. Subjects were presented with the three odorless stimuli (PEA-, EUG- and PG) twice to each nostril. They were not able to identify them above the 33% chance level [$t(10) = 1.14$, $1.68$ and $1.02$ for PEA-, EUG- and PG, respectively, $p > .05$].

Each subject performed 10 localization tasks, half of which were presented in the binaral odorant condition where two different odorants were inhaled one to each nostril (PEA+/EUG-, PEA+/EUG+, PEA++/EUG+, EUG+/PEA-, EUG++/PEA+) and half were
presented in the mononaral odorant condition where a single odorant was inhaled via either one or both nostrils (PEA+/PEA-, PEA+/PG, PEA++/PG, EUG+/PG, EUG++/PG). Each task consisted of 2 blocks of 20 trials that were conducted at the same time of the day 1 to 2 days apart from one another. Subjects completed 5 tasks per day with a 10 min break in between the tasks. Each trial started with 2 low beeps and 1 high beep (Fig. 1).

The experimenter presented a pair of smells on the third beep. Subjects were instructed to exhale through the mouth upon the first low beep and inhale through the nosepieces upon the third beep. Subsequently, they pressed one of two keys to indicate whether the target smell came from the left or right nostril. The target smell was presented to left and right nostril equally within a block. The orders of the task and nostril side where the target smell was presented were randomized. At the beginning and the end of each block, subjects’ left and right nasal airflow rates were acquired using rhinospirimeter (NV1; GM Instruments Ltd, Kilwinning, UK). At the conclusion of each block, subjects provided their level of confidence in their localization accuracy. At the conclusion of the entire experiment, 11 out of 12 subjects rated the perceived intensity of the target smell on each task on a 100-point visual analog scale (VAS). The bottles were labeled with numbers. Both the experimenters and subjects were blind to the purpose of the experiment, the smell condition and the side of the nostril in which the target smell was presented.

![Fig. 1. Illustration of an experimental trial.](image)

Subjects exhaled at the prompt of the first beep, took a single inhalation of a pair of odorants at the end of the third beep, and
indicated with button pressing the location of the odorant. Subjects inhaled through the nose and exhaled through the mouth throughout the experiment.

**Data analyses**

Nasal airflow asymmetry index (AI) was calculated by dividing the mean left nostril airflow rate by the mean right nostril airflow rate (J. Porter et al., 2005). Pearson’s r correlation coefficient and paired-sample t tests were used to estimate the reliability of localization accuracies, AI and level of confidence in localization response for the same localization task across blocks.

Because the localization accuracy, AI and level of confidence for the same task were significantly correlated between the blocks (r = 0.52, 0.68 and 0.69 respectively, ps < 0.001) and were not significantly different from one another [t(119) = 0.23, 0.72 and 0.94 for localization accuracy, AI and level of confidence, ps > 0.05], localization accuracies were combined and compared with 50% chance level using one-sample t tests with Bonferroni adjustment for multiple comparisons (Bonferroni corrected α = 0.005). AI and level of confidence were also averaged across two blocks and subjected to Pearson’s correlation with localization accuracy.

Paired sample t tests were further used to analyze perceived rose intensity of PEA+/EUG- vs. PEA+/PG and perceived clove intensity of EUG+/PEA- vs. EUG+/PG.

**Results and Discussion**

*Maximal contrast between nasal passages enables olfactory localization*

Subjects localized the target rose smell significantly above chance in the binaral odorant condition when they smelled a detectable and just identifiable smell (PEA+) in
one nostril, and an undetectable, unidentifiable, and different (EUG-) smell in another nostril \[ t(11) = 7.34, p < 0.001; \text{Fig. 2}. \]

**Intensity contrast alone is not sufficient for olfactory localization**

We asked if localization could be achieved in the mononaral odorant condition when only a single odorant was presented to the nostrils. We tested this by presenting a detectable and just identifiable PEA+ in one nostril and an undetectable and unidentifiable PEA- in the other nostril. Subjects did not localize the rose smell \[ t(11) = 0.40, p = 0.70; \text{Fig. 2}, \] indicating that intensity contrast alone is not sufficient for successful localization of smells.

**Quality gradient alone is not sufficient for olfactory localization**

The localization of the rose smell in the PEA+/EUG- pair could be due to mere quality difference between the rose and the clove smell. To test this, we presented subjects with a pair of smells that differed in quality (PEA+ vs. EUG+) but were equally intense and identifiable. Subjects did not localize the rose smell \[ t(11) = 0.28, p = 0.79; \text{Fig. 2}, \] indicating that mere quality difference is not sufficient to produce localization.

This lack of localization could be due to insufficient intensity/identifiability contrast between the nostrils. We subsequently presented subjects with a clearly detectable and identifiable rose smell (PEA++) and a detectable and just identifiable clove smell (EUG+). Subjects still did not localize the rose smell \[ t(11) = 0.90, p = 0.39; \text{Fig. 2}, \] showing that a combination of quality and intensity gradients is not sufficient to produce localization.
Successful localization is not due to synergistic interaction between inputs to the nostrils

Localization of the rose smell could be due to synergistic interactions (Smith, 1998) between a suprathreshold PEA and a subthreshold EUG. If this were the case, the perceived intensity of the rose smell in the PEA+/EUG- condition would be different from that in the PEA+/PG condition. However, we assessed subjects’ perceived intensity of the target smell and found that PEA+/EUG- and PEA+/PG did not differ by perceived intensity \( t(10) = 1.68, p = 0.12; \) Fig. 2.

Successful localization is not due to trigeminal sensation

To confirm that PEA is a pure odorant (Doty et al., 1978), we performed the trigeminal paradigm (Frasnelli et al., 2009, 2010; Kleemann et al., 2009; Kobal et al., 1989; Radil & Wysocki, 1998; Schneider & Schmidt, 1967; Wysocki et al., 2003), in which one of subjects’ nostrils was presented with the detectable and just identifiable smell (PEA+) or clearly detectable and identifiable smell (PEA++) and the other nostril was presented with the diluent (PG). Subjects failed to localize the rose smell in PEA+/PG and PEA++/PG concentrations \( t(11) = 2.28 \) and \( 3.39 \) for PEA+ and PEA++, respectively, \( ps > 0.005; \) Fig. 2], confirming that the PEA is a pure odorant and successful localizing the rose smell is not due to trigeminal sensation.
Fig. 2. Localization of the rose smell. (A) Subjects successfully localized the rose smell above the 50% chance level when they were binaurally presented with a distinctive target rose smell (PEA+) in one nostril and a weak, different, and unidentifiable smell (EUG-) in the other nostril. (B) Perceived intensity did not differ significantly between PEA+/EUG- and PEA+/PG conditions. The asterisks, dash line and error bars represent p < 0.001, 50% chance level and standard errors of the mean, respectively.

Successful localization is not due to asymmetric airflow

Successful localization could be due to asymmetric airflow between the nostrils (Klein, Pilon, Prosser, & Shannahoff-Khalsa, 1986). Yet, subjects’ nasal airflow asymmetry did not correlate significantly with localization accuracy (r = -0.08, p = 0.51; Fig. 3A).

Successful localization is not due to cognitive strategies

Successful localization in the PEA+/EUG- condition could be due to the tactile sensation of the nostril that received the smell, which, in turn, may enable subjects to form effective cognitive strategies. We therefore asked subjects to indicate the level of confidence in their responses at the end of each block. Subjects’ level of confidence was
not related to their localization accuracy ($r = -0.22$, $p = 0.07$; Fig. 3B), and if anything, there is a marginally significant negative correction between level of confidence and localization accuracy. Those who were more confident tended to be less accurate.

**Fig. 3. Successful localization of the rose smell is not due to asymmetric airflow or cognitive strategies.** There were no significant correlations between localization accuracy of rose smell and asymmetry index, and between localization accuracy of rose smell and level of confidence. The dash lines represent the best-fit curves.

**Successful localization is replicated with a different target smell**

Finally, our finding could be unique to the rose smell used in the study. To examine this, we reversed the order of the target smell so that subjects were asked to localize the clove smell. Using the same paradigm, we replicated our findings that subjects only localized the clove smell when they smelled a detectable and identifiable smell (EUG+) in one nostril and an undetectable and unidentifiable and different smell (PEA-) in another nostril [$t(11) = 6.94$, $p < 0.001$; Fig. 4]. EUG is not a trigeminal smell because subjects did not localize it in the two suprathreshold concentrations [$t(11) = 3.08$ and $1.32$ for EUG+ and EUG++, respectively, $ps > 0.005$; Fig. 4]. As with before, the intensity of EUG+/PEA- and EUG+/PG tasks were perceived to differ significantly [$t(10) = 0.12$, $p =
Nor was their localization accuracy related to AI ($r = 0.01, p = 0.96$) and level of confidence ($r = -0.10, p = 0.50$).

**Fig. 4. Localization of the clove smell.** (A) Subjects successfully localized the clove smell in the context of EUG+ in one nostril and PEA- in the other nostril, replicating the results in localization of the rose smell. (B) Perceived intensity did not differ significantly between EUG+/PEA- and EUG+/PG conditions. The asterisks, dash line and error bars represent $p < 0.001$, 50% chance level and standard errors of the mean, respectively.

Lateral inhibition increases contrast and sharpens neural response in vision (Blakemore & Tobin, 1972; Nordström & O’Carroll, 2009) and audition (Grothe, 2003; Pecka, Brand, Behrend, & Grothe, 2008), and is likely to underlie the olfactory localization observed here. Neuroanatomically, olfactory information projects from each nostril to its ipsilateral hemisphere, and only crosses over in the anterior olfactory nucleus (AON) via the anterior commissure (AC) (Kikuta et al., 2010; Yan et al., 2008; Brunjes et al., 2005; Wilson, 1997). When both nostrils are simultaneously stimulated, inputs to the contralateral nostril elicit inhibitory responses, causing reduction of the magnitude of
neural response in the ipsilateral nostril (Kikuta et al., 2010), enhancing the contrast between olfactory percepts in the two nostrils and enabling stereo localization of odors.

Human perception is multisensory where spatial information can be provided by redundant sensory cues. We show previously that olfaction and vision integrate in a nostril specific fashion (Zhou, Zhang, Chen, Wang, & Chen, 2012). The egocentric localization of olfactory cues observed here may have arisen from the need to quickly calibrate and extract spatial cues from different senses.

**Conclusion**

Spatial localization can be allocentric (relative to objects in space) or egocentric (relative to oneself). We demonstrate that humans use a combination of intensity, identifiability, and quality contrasts between olfactory inputs to the nostrils to egocentrically localize smells. We show this ability is independent of conscious verbal awareness. We rule out trigeminal, tactile, and synergistic interactions between the smells as alternative explanations. Our findings add to the literature on spatial localization and shed new light on the mechanism of olfactory perception.
STUDY III: NOSTRIL-SPECIFIC OLFATORY MODULATION OF VISUAL PERCEPTION

Introduction

Both olfaction and vision serve the function of object identification. Visual cues are known to facilitate the detection of congruent odorants, and such enhancement has been proposed to be mediated by mnemonic processes based on their semantic associations (Gottfried & Dolan, 2003). Likewise, olfaction modulates visual object perception, even in the absence of conscious visual awareness (Zhou et al., 2010). Yet it remains unclear at which stages of the sensory processing hierarchy the two types of inputs converge, despite recent advances in our understandings of multisensory regions and multisensory integration (Beauchamp, 2005; Macaluso & Driver, 2005; Stein & Stanford, 2008). At first glance, olfaction and vision are anatomically distant, with primary olfactory areas situated in the inferior frontal and anterior temporal regions, and primary visual areas in the occipital lobe. Primary olfactory projections are largely ipsilateral, from the olfactory epithelium in one nostril to the olfactory bulb and then the anterior olfactory nucleus, olfactory tubercle, piriform, amygdala, and entorhinal cortex on the same side, with only slight projection to the contralateral side by way of the anterior commissure (Powell et al., 1965; Price, 1973). By contrast, primary visual projections are mainly contralateral: inputs from the left or right visual field are transferred to the striate and extrastriate cortices on the opposite side (DeYoe et al., 1996). Further downstream, there are category-selective regions including the left-lateralized visual word form area (VWFA) (McCandliss, Cohen, & Dehaene, 2003) and the right-lateralized extrastriate body area
(EBA) and fusiform body area (FBA) (Downing, Jiang, Shuman, & Kanwisher, 2001; Schwarzlose, Baker, & Kanwisher, 2005; Willems, Peelen, & Hagoort, 2010), that selectively respond to words and human bodies, respectively. Taking advantage of such anatomical and functional lateralizations in the olfactory and visual systems, we carry out three experiments to probe the aforementioned issue of where the two senses converge. We do so utilizing a well-established visual phenomenon termed binocular rivalry – perceptual alternations that occur when distinctively different images are separately presented to the two eyes (Blake & Logothetis, 2002) - a paradigm that has proven sensitive to the interplays between vision and other senses (Lunghi, Binda, & Morrone, 2010; van Ee, van Boxtel, Parker, & Alais, 2009; Zhou et al., 2010).

Materials and Methods

Participants

A total of 82 healthy right-handers with normal or corrected-to-normal vision participated in the study; 24 (10 males, mean age = 21.8 yrs, SEM = 0.35) took part in Experiment 1, 30 (11 males, mean age = 22.1 yrs, SEM = 0.86) in Experiment 2, and 28 (10 males, mean age = 24.2 yrs, SEM = 0.40) in Experiment 3. At the time of testing, all subjects reported to have normal sense of smell and no respiratory allergy or upper respiratory infection. They gave informed consent for participation and were unaware of the purposes of the experiments.

Visual stimuli

All visual stimuli were displayed on a 19” flat screen monitor, dichoptically presented to the two eyes, and engaged in rivalry. We individually adjusted which eye viewed
which image to produce a more balanced rivalry between the competing images in the absence of olfactory cues. In Experiment 1, two colored images of a rose and a banana, respectively, were displayed side by side and fused with a mirror stereoscope mounted to a chinrest (visual angle = 1.7°×2.2°, with the center 1.3° horizontally from the fixation either in the left or the right visual field, Fig. 1A), such that the rose image was presented to the left or right visual field of one eye while the banana image was presented to the same visual field of the other eye. To facilitate stable convergence of the two eyes’ images, each image was enclosed by an identical square frame (10.7°×10.7°) centered on the fixation cross. In Experiment 2, a composite image of words in green and a human body in red (visual angle = 2.7°×3.2°) was shown at the center of the monitor and viewed through red-green anaglyph glasses, so that the words were presented to the central visual field of one eye while the human body was presented to that of the other eye (Fig. 2A). We chose to use red-green anaglyph glasses instead of mirror stereoscope as it produced a more balanced rivalry between the relatively low contrast human body image and the relatively high contrast words image with adjustments of their colors, without making the images look unnatural to the observers. Experiment 3 adopted the same visual stimulation setup as in Experiment 1 except that the rose image was replaced with an image in which the word ‘rose’ was repeated four times (1.7°×2.2°) and the two competing images were respectively presented to the central visual field of each eye (Fig. 3A).

Olfactory stimuli

The olfactory stimuli in Experiments 1 and 3 consisted of phenyl ethyl alcohol (PEA, a rose-like smell, 0.5% v/v in propylene glycol) and isoamyl acetate (IA, a banana-like smell, 0.02% v/v in propylene glycol). In addition, purified water was used to achieve
unilateral smell presentation. These were presented in identical 20ml polypropylene jars. Each jar contained 10 ml clear liquid and was fitted with a Teflon nosepiece. The olfactory stimuli in Experiment 2 consisted of PEA (1% v/v in propylene glycol), n-butanol (a marker-pen like smell, 0.5% v/v in propylene glycol), and natural human body odor (pooled sweat collected from three male donors aged 20, 23, and 24, who kept a 4”×4” nylon/polyester blended pad under each armpit for two hours when performing non-strenuous daily activities). Purified water was also used to achieve unilateral smell presentation. These stimuli were presented on nylon/polyester-blended pads (4”×4”) in identical 40ml polypropylene jars, each fitted with a Teflon nosepiece. To form a single “pooled sweat pad”, roughly the 1/3 of layers closest to the skin during sweat collection were taken from one of each donor’s pads and mixed together. For PEA, butanol, and water, 1ml of each was respectively applied to a different pad and placed in a separate jar. Detailed procedures for sweat collection and storage have been described elsewhere (Zhou & Chen, 2009b).

In each experiment, the subjects held two jars (one containing a smell and the other containing purified water) with their left hand and positioned the nosepieces into the two nostrils as instructed by the experimenter. They were told to continuously inhale through the nosepieces and exhale through their mouth. This method is standard in the field to achieve unilateral olfactory stimulation (Wysocki et al., 2003). All olfactory stimuli were supra-threshold to all the subjects.

**Procedure**

The subjects in Experiments 1 and 3 firstly sampled the olfactory stimuli with both nostrils, one at a time in a randomized order. After the sampling of each stimulus, they
rated its intensity, pleasantness, as well as similarities to the smells of rose and banana, respectively, on a 100-unit visual analogue scale. There was at least a one-minute break in between the samplings. After the olfactory stimuli assessment, the experimenter individually adjusted the mirror stereoscope for each subject to ensure binocular fusion. The subjects then completed a practice session so that they were comfortable with viewing the images through the mirror stereoscope and maintaining their fixation at the central fixation point while continuously inhaling through their nose and exhaling through their mouth. They were instructed to press one of two buttons with their right hand when they saw predominantly ‘rose’ (rose image in Experiment 1 and rose word in Experiment 3), and press the other button when it switched to predominantly ‘banana’. The button presses marked the time points of perceptual switches. Each subject in Experiment 1 completed the actual binocular rivalry task eight times, each time with a different combination of olfactory stimulus (PEA or IA), nostril side (smelling the olfactory stimulus in the left or the right nostril), and rivalry visual field (binocular rivalry taking place in the left or the right visual field). Those in Experiment 3 viewed the competing images in the central visual field and completed the actual binocular rivalry task four times, each time with a different combination of olfactory stimulus (PEA or IA) and nostril side (smelling the olfactory stimulus in the left or the right nostril). Each run lasted 60s, with a 3-minute break in between the runs. The order of the conditions was randomized and balanced across the subjects. At the end of each run, the subjects reported which nostril they thought received a smell. No feedback was provided during the experiment.
Experiment 2 followed similar procedures as in Experiments 1 and 3, except that red-green anaglyph glasses were used. The subjects assessed the intensity and pleasantness of each olfactory stimulus and verbally described what each smelled like before performing the binocular rivalry task, in which they pressed one of two buttons when they saw predominantly ‘words’, and pressed the other button when the percept switched to predominantly ‘human body’. The two competing images were centrally fixated. There were a total of six 60s runs, each with a different combination of olfactory stimulus (PEA, butanol, or natural human body odor) and nostril side (smelling the olfactory stimulus in the left or the right nostril). The order of the runs was randomized and balanced across the subjects, and there was a 3-minute break in between the runs.

Data analyses

For each condition, we firstly calculated the mean duration (d) that one image predominated over the other, namely, the averaged duration between pressing one button for beginning to see predominantly one of the rivalry images and pressing the other button for beginning to see predominantly the other rivalry image. This was then converted to the proportion (prop) that one image predominated over the other, and used as our dependent measure. For example, in Experiments 1 and 3, the proportion that the rose image (Experiment 1) or rose word (Experiment 3) predominated over the banana image \( \text{prop}_{\text{rose}} \) was calculated as: 
\[
\text{prop}_{\text{rose}} = \frac{d_{\text{rose}}}{d_{\text{rose}} + d_{\text{banana}}}.
\]
Correspondingly, \( \text{prop}_{\text{banana}} = 1 - \text{prop}_{\text{rose}} \). In Experiment 2, we specifically used the proportion that the body image predominated over the words as the dependent measure.

The data were analyzed with repeated measures ANOVA, using olfactory stimulus (PEA vs. IA), nostril side (left nostril vs. right nostril), and visual field (left visual field
vs. right visual field) as the within-subject factors in Experiment 1; olfactory stimulus (PEA vs. butanol vs. natural body odor) and nostril side (left nostril vs. right nostril) as the within-subject factors, and sweat identification (describing the sweat samples as human-related vs. as other non-biological objects) as the between-subjects factor in Experiment 2; olfactory stimulus (PEA vs. IA) and nostril side (left nostril vs. right nostril) as the within-subject factors in Experiment 3. In Experiments 2 and 3, paired sample t tests were further performed for each olfactory stimulus to compare the dominance proportion of a rivalry image when smelling it from the left versus the right nostril.

Results and Discussion

**Nostril- and visual field- specific olfactory modulation of visual perception in binocular rivalry**

In Experiment 1, two images of rose and banana were engaged in binocular rivalry either in the left or the right visual field (Fig. 1A) while the subjects were being exposed continuously to PEA or IA in one of the two nostrils, and purified water in the other nostril. As compared with IA, PEA was rated as much more like the smell of rose (p < 0.001), much less like the smell of banana (p < 0.001), but equally intense (p = 0.38) and pleasant (p = 0.20). Overall, the rose image was dominant in view for longer when the subjects smelled PEA relative to IA [F (1,23) = 10.77, p = 0.003, Fig. 1B], and vice versa, replicating an earlier finding (Zhou et al., 2010). Critically, this effect varied with whether the side of the nostril that received PEA or IA was contralateral or ipsilateral to the visual field where the rivalry took place [F (1,23) = 10.57, p = 0.004, Fig. 1B].
Smelling PEA from the contralateral relative to the ipsilateral nostril significantly increased the proportion that the rose image was dominant \( [F(1,23) = 4.49, p = 0.045, \text{Fig. 1C}] \), and smelling IA from the contralateral relative to the ipsilateral nostril significantly increased the proportion that the banana image was dominant \( [F(1,23) = 4.63, p = 0.042, \text{Fig. 1D}] \). The effect was observed even though the subjects were unaware of which nostril received an odorant (mean accuracy = 0.48 and 0.44 for PEA and IA, respectively; versus chance = 0.50). As primary olfactory regions receive mainly inputs from the ipsilateral nostril (Powell et al., 1965; Price, 1973) and early visual areas receive mainly inputs from the contralateral visual field (DeYoe et al., 1996), these results show a clear within-hemisphere advantage (Heilige, 1993) in the integration of olfactory and visual information that occurs relatively early in the sensory processing hierarchy.

**Fig. 1. Nostril- and visual field-specific olfactory modulation of visual perception in binocular rivalry.** (A) Visual stimuli used in Experiment 1 were viewed through mirror stereoscope and dichoptically presented to the left eye (LE) and the right eye (RE), with fused images of rose and banana in either the left (LVF) or the right visual field (RVF). (B) On top of an overall enhancement of the congruent image’s dominance over the incongruent one, the dominance proportion of an image depended on both the input odorant and the nostril receiving that odorant. (C) Relative to the ipsilateral nostril,
smelling PEA in the nostril contralateral to the rivalry site increased the dominance of the rose image. (D) Relative to the ipsilateral nostril, smelling IA in the nostril contralateral to the rivalry site increased the dominance of the banana image. Error bars represent standard errors of the mean, adjusted for individual differences. Error bars shorter than the diameter of the markers are not displayed.

**Nostril-specific olfactory modulation of category-selective visual processing**

We went on to examine whether such nostril-specific effect persists in the downstream category-selective areas. Experiment 2 introduced three smells – PEA, n-butanol, and natural human body odor, each presented in a unilateral manner as in Experiment 1 – to the binocular rivalry between two images of words and human body in the fovea (Fig. 2A). The three odorants were matched in intensity (p = 0.18). Butanol and body odor were rated as equally unpleasant (p = 0.17), and significantly less pleasant than PEA (ps < 0.001). The subjects did not know which nostril received an odor throughout the experiment (mean accuracy = 0.52, 0.58, and 0.48 for body odor, PEA, and butanol, respectively; versus chance = 0.50). Whereas generally speaking smelling natural human body odor increased the proportion that the body image was dominant in view [F(2, 56) = 3.80, p = 0.028] regardless of whether the subjects were verbally aware of the nature of the odorant [F(1, 28) = 0.37, p = 0.55], smelling it from the right nostril led to a greater increase relative to the left nostril [t(29) = 2.16, p = 0.039], an effect not found with PEA (p = 0.93) or butanol (p = 0.84) (Fig. 2B). These results again reflect a within-hemisphere advantage in the integration of the two senses further down the visual processing hierarchy, since words and human bodies, though engaged in binocular rivalry in the central visual field, are processed in visual word form area (McCandliss et al., 2003) and body-selective regions (Downing et al., 2001; Schwarzlose et al., 2005; Willems et al., 2010) lateralized to the left and the right hemisphere, respectively.
Fig. 2. Nostril-specific olfactory modulation of category-selective visual processing.

(A) Visual stimuli used in Experiment 2 were viewed through red/green anaglyph glasses and dichoptically presented to the two eyes, with fused images of words and human body in the central visual field. (B) Compared with butanol and PEA, smelling human body odor increased the proportion that the body image was dominant in view, and such increase is more pronounced when the smell was sampled from the right nostril relative to the left nostril. Error bars represent standard errors of the mean, adjusted for individual differences.

Nostril-specific olfactory modulation of visual processing depends on sensory rather than semantic congruency

Experiment 2 does not address if category selective processing of word forms in the left hemisphere also benefits from a semantically congruent odor in the left as opposed to the right nostril. This was tested in Experiment 3 with a similar design to that of Experiment 2. The same olfactory stimuli as in Experiment 1 were used, but the rose image was replaced with an image where the word ‘rose’ was repeated four times (to form a global shape roughly matching that of the banana image and facilitate binocular rivalry). As in Experiment 2, the two images (an image of banana and an image of the word ‘rose’) were engaged in binocular rivalry in the central visual field (Fig. 3A) while
the subjects smelled PEA or IA in either the left or the right nostril (see Materials and Procedure for details). PEA was again perceived to be more like the smell of rose (p < 0.001) and less like the smell of banana (p < 0.001) relative to IA, but equally intense (p = 0.25) and pleasant (p = 0.10). Similar to Experiments 1 and 2, the subjects did not know to which nostril an odor was being presented (mean accuracy = 0.48 and 0.46 for PEA and IA, respectively; versus chance = 0.50). Here we observed a main effect of smell, such that the word ‘rose’ was dominant in view for longer when the subjects smelled PEA as compared with IA, and vice versa [F(1,27) = 4.92, p = 0.035 in both cases]; yet there was no nostril difference for either smell [t(27) = 0.008 and -0.55, p = 0.99 and 0.59 for PEA and IA, respectively] (Fig. 3B). The visual processing of the centrally presented banana image is not lateralized, thus smelling IA in either nostril was expected to produce comparable effects in boosting the dominance of the banana image. However, smelling PEA in the left nostril did not preferentially enhance the dominance of the word ‘rose’ relative to the right nostril, despite that the neural representations of the visual word form and the semantic meaning of ‘rose’ are both left lateralized (Frost, 1999; McCandliss et al., 2003). We therefore concluded that nostril-specific olfactory modulation of visual processing, as observed in Experiments 1 and 2, relied not only on the anatomical and functional lateralizations in the two systems, but also on the sensory rather than semantic congruency between olfactory and visual inputs.
Fig. 3. Nostril-specific olfactory modulation of visual processing depends on sensory rather than semantic congruency. (A) Visual stimuli used in Experiment 3 were viewed through mirror stereoscope and dichoptically presented to the left eye (LE) and the right eye (RE), with fused images of rose word and banana in the central visual field. (B) Compared with IA, smelling PEA increased the dominance of the rose word with no difference between the two nostrils. Error bars represent standard errors of the mean, adjusted for individual differences.

The human brain is wired to efficiently coordinate the senses and integrate their inputs. In the case of olfaction and vision, both capturing the identities of objects, it is commonly held that they interact in a top-down manner at the semantic level with olfaction frequently succumbing to visual modulations (Gottfried & Dolan, 2003; Morrot et al., 2001). Whereas the current study by no means negates this account, it has shed new light into the basic neural substrates underlying olfactory-visual integration by taking advantage of the anatomical and functional lateralizations in the two systems. We observe a nostril-specific olfactory modulation of binocular rivalry for processes in early visual cortices as well as category selective visual regions based on sensory rather than semantic congruency. Such nostril-specific modulation cannot be due to top-down attentional or cognitive control as the subjects were unaware of which nostril received an
odorant. It was also highly unlikely that they knew about the lateralizations in both the visual and the olfactory systems. Our results thus indicate that olfactory and visual integration occurs at the stage of sensory representations early in the information processing hierarchy. There information from the two sources is automatically assembled in an object-based manner (Experiments 1 & 2), independent of object identification or semantic processing at the conscious level (Experiments 2 & 3) (Zhou et al., 2010). In doing so, they provide strong human behavioral evidence for multisensory integration in relatively early sensory cortices.

Moreover, while a large body of literature exists on visual hemifield and retinotopic mappings, there has only been very limited research on the functional relevance of the ipsilateral primary olfactory projections (J. Porter et al., 2007; Zhou & Chen, 2009c). By highlighting the functional dissociation of the two nostrils, our findings narrow this gap.

Recent animal studies have outlined direct connections among primary auditory, visual, and somatosensory cortices (Falchier, Clavagnier, Barone, & Kennedy, 2002; Fu et al., 2003; Iurilli et al., 2012; Wallace, Ramachandran, & Stein, 2004). It has also been proposed that associative neuronal plasticity prevails in early sensory cortices, possibly involving a Hebbian mechanism for enhancement of synaptic efficacy (Albright, 2012). Whereas the anatomical connectivity between olfactory and visual regions remains poorly understood, convergent projections from the retina and from the olfactory bulbs have been observed in the olfactory tubercle and piriform cortex in a range of mammalian species including primates (Cooper et al., 1994; Mick et al., 1993; Pickard & Silverman, 1981). In humans, individual differences in the nasal cycle and binocular rivalry alternation rate are correlated, pointing to an endogenous shared mechanism regulating
both the olfactory and the visual systems (Pettigrew & Carter, 2005). Furthermore, a latest study showed that repetitive transcranial magnetic stimulation of V1 enhances odor quality discrimination (Jadauji, Djordjevic, Lundström, & Pack, 2012). The exact signaling pathways mediating the observed early convergence of olfactory and visual information await future studies.

Conclusion

We show that smelling an odor from one nostril significantly enhances the dominance time of the congruent visual image in the contralateral visual field, relative to that in the ipsilateral visual field. Moreover, such lateralization-based enhancement extends to category selective regions so that when two images of words and human body, respectively, are engaged in rivalry in the central visual field, smelling natural human body odor from the right nostril increases the dominance time of the body image compared with smelling it from the left nostril. Semantic congruency alone failed to produce this effect in a similar setting. These results, taking advantage of the anatomical and functional lateralizations in the olfactory and visual systems, highlight the functional dissociation of the two nostrils and provide strong evidence for an object-based early convergence of olfactory and visual inputs in sensory representations.
STUDY IV: THE DUAL FUNCTION OF BASIC TASTE STIMULI: SIGNALING NUTRIENTS IN SMELL AND TASTE

Introduction

Monosodium glutamate (MSG), sucrose, citric acid, sodium chloride (NaCl), and quinine are known to impart five basic taste sensations – umami, sweet, sour, salty and bitter – and signal nutritional values of the food – protein, energy, spoiled food, sodium and toxins, respectively (X. Chen, Gabitto, Peng, Ryba, & Zuker, 2011). In fact, studies show that taste guides food selection and promotes the ingestion of nutrients (Cassady & Mattes, 2010). For example, infants consume less formula with higher protein to satiation (Ventura, Beauchamp, & Mennella, 2012); college-age subjects are hungrier and consumed greater amount of carbohydrates after 2-hour-long exercise (Verger, Lanteaume, & Louis-Sylvestre, 1992). Obese women have lower taste sensitivity to MSG and prefer soup with higher MSG concentrations (Pepino, Finkbeiner, Beauchamp, & Mennella, 2010). A positive correlation between the amount of MSG intake and body mass index (BMI) is observed in Chinese adults (He et al., 2008). Obese individuals also find sugar less sweet and prefer it more than non-obese individuals (Bartoshuk, Duffy, Hayes, Moskowitz, & Snyder, 2006).

The evidence of olfactory sensing of nutrients in basic tastants are derived predominantly from animal studies. The latency of the rats’ first lick of the sucrose solution decreases proportionally to its concentration (Rhinehart-doty, Schumm, Smith, & Smith, 1994). Rats consume less NaCl solutions after being conditioned to its odor (Capaldi, Hunter, & Privitera, 2004; Privitera & Capaldi, 2006). Dissecting rat’s olfactory
nerve reduces the discrimination accuracy of NaCl solution versus distilled water to chance level (Miller & Erickson, 1966). Olfactory bulbectomy diminishes consumption of dilute sucrose solution in rats (Zukerman, Touzani, Margolskee, & Sclafani, 2009) and averseness to NaCl solutions in sheep (Bell, Dennis, & Sly, 1979). There is reason to believe that humans likewise detect the smell of basic tastants. Perceived intensity of umami taste in the mouth is significantly reduced when subjects wear a nose clip, implying that orthonasal sensing contributes to the retronasal detection of tastes (Mojet, Köster, & Prinz, 2005). Henkin and his colleagues report that humans, particularly patients of cystic fibrosis, smell sucrose and NaCl orthonasally (Henkin, Gill, & Batter, 1962; Henkin & Powell, 1962). Another study finds orthonasal detection of NaCl in only 1 cystic fibrosis patient (Hertz, Cain, Bartoshuk, & Dolan, 1975). While some studies attribute the findings to potential impurities in the taste compound (Miller & Erickson, 1966; Mojet et al., 2005), other studies argue against the impurity explanation by showing similar responses to the smell of reagent and food grades tastants (Rhinehart-doty et al., 1994; Zukerman et al., 2009).

Molecular evidence similarly favors the notion of olfactory sensing of tastants. A variant subfamily of Ionotropic glutamate receptors (iGluRs), termed Ionotropic Receptors (IRs), is found to be expressed in the olfactory organs of the fruit fly (Abuin et al., 2011; Benton, Vannice, Gomez-Diaz, & Vosshall, 2009) and Protostomia (Croset et al., 2010). Furthermore, taste-like receptors are widely distributed in not only the mouth, but also other regions of the body, including the nasal cavity, gut, and large intestine, where they no longer reflect the taste qualities, but nevertheless regulate food ingestion
and digestion (Finger & Kinnamon, 2011; Tizzano, Cristofoletti, Sbarbati, & Finger, 2011).

In addition to the evidence on the olfactory sensing of tastants, there is reason to believe that homeostatic states regulate olfactory sensitivity to food related smells. The level of endocannabinoid 2-arachidonoyl-glycerol (2-AG), an intercellular lipid messenger in the central nervous system and olfactory epithelium, rises in hungry animals and sharpens their olfactory sensitivity (Breunig, Czesnik, et al., 2010; Breunig, Manzini, et al., 2010; Palouzier-Paulignan et al., 2012). 2-AG antagonists, on the other hand, suppress the neural responses to olfactory stimuli (Czesnik, Schild, Kuduz, & Manzini, 2007; Sink, Vemuri, Olszewska, Makriyannis, & Salamone, 2008). Odor maps in the olfactory bulbs change in accord with the hunger state in the Drosophila (Root, Ko, Jafari, & Wang, 2011). Moreover, mitral cell responses to food odor are enhanced in rats after food deprivation, reduced after satiation, and show no change to non-food odors across the two sessions (Pager, Giachetti, Holley, & Le Magnen, 1972; Pager, 1974). On the neural endocrine level, receptors of ghrelin, an appetite-stimulating hormone, are identified in the olfactory bulb. Increasing the level of ghrelin lowers olfactory thresholds and enhances exploratory sniffing magnitude to both food and nonfood smells in rodents and humans (Palouzier-Paulignan et al., 2012; Tong et al., 2011). Another study reports that the receptors of orexins, a neuropeptide responsible for regulating food intake and homeostatic states, are widely distributed from peripheral to cortical levels in the olfactory system (Caillol, Aïoun, Baly, Persuy, & Salesse, 2003). Specifically, stimulation with orexin leads to hunger sensation and higher olfactory sensitivity (Apelbaum, Perrut, & Chaput, 2005; Julliard et al., 2007; Palouzier-Paulignan et al.,
Finally, neural responses in the orbitofrontal cortex are less reactive to the food smell (e.g., banana) that animals consume to satiety (e.g., a meal of bananas), but remain unchanged toward the smell (e.g., vanilla) of food that the animals did not consume in the meal (Critchley & Rolls, 1996; O’Doherty et al., 2000). Behavioral evidence, however, is mixed. Food smells are perceived as more pleasant when individuals are hungry than satiated (Cabanac, 1971; Plailly et al., 2011). Some studies show increased olfactory sensitivity when individuals are hungry (i.e., before a meal or after fasting) (Aimé et al., 2007; Goetzl, Abel, & Ahokas, 1950; Goetzl & Stone, 1947; Hammer, 1951; Schneider & Wolf, 1955; Stafford & Welbeck, 2011) while other studies find either the opposite effect (i.e., lower olfactory sensitivity before a meal or after fasting) (Albrecht et al., 2009; Berg, Pangborn, Roessler, & Webb, 1963), or no change between hungry and satiated states (Furchtgott & Friedman, 1960; Janowitz, 1949; Koelega, 1994; Zilstorff-Pedersen, 1955). Some of the discrepancies may be explained by confounding order effect (Stafford & Welbeck, 2011). In addition, the interaction between the energy content of the food and the physiological states of hunger and satiety may affect neural responses to sensory stimuli (Haase, Cerf-Ducastel, & Murphy, 2009; Siep et al., 2009); since the smells and homeostatic states vary by studies, the results can be mixed.

Here, we perform psychophysical tests to determine whether humans detect the smell of basic tastants, which are commonly believed to be odorless (Experiment 1), and to investigate whether the detection was due to the impurities in the tastant (Experiment 2). Having established that the humans can “smell” tastants, we next asked whether sensitivity to the smell can be modulated by homeostatic states (Experiment 3). We next probed the function of olfactory sensing of taste by subjecting the smell and taste of the
tastants to the binocular rivalry paradigm, to assess whether the smell of tastants facilitated visual detection of nutritionally congruent food (Experiment 4).

**Materials and Methods**

**Participants**

30 (11 males, 19 females; mean age = 23.17, SEM = 0.72; mean BMI = 24.56, SEM = 0.74, range 18.6-34.6), 10 (5 from Experiment 1; 7 males, 3 females; mean age = 23.00, SEM = 1.51; mean BMI = 23.63, SEM = 0.83, range 20.6-27.3), 15 (12 from Experiment 1; 9 males, 6 females; mean age = 23.67, SEM = 1.12; mean BMI = 24.29, SEM = 1.03, range 19-34.6), and 30 (8 from Experiment 1; 12 males, 18 females; mean age = 24.73, SEM = 0.65; mean BMI = 23.51, SEM = 0.61, range 16.5-30.6) subjects participated in Experiment 1, 2, 3 and 4, respectively. All subjects were healthy non-smokers, reporting normal sense of smell and taste, and no rhinal problems. Eligible subjects in Experiment 4 were also required to have normal or correct to normal vision.

**Olfactory stimuli**

5 reagent grade (Sigma-Aldrich) tastants were diluted in double-distilled deionized water, making 2.36 M MSG (umami; ≥ 99% purity), 1.87 M sucrose (sweet; ≥ 99% purity), 2.26 M citric acid (sour; ≥ 99.5% purity), 2.86 M NaCl (salty; ≥ 99.5% purity) and 0.05 M quinine monohydrochloride dihydrate (bitter; 90% purity) in Experiment 1. Blank was double-distilled deionized water. Each olfactory stimulus (10 ml) was contained in a 280 ml glass bottle that was fitted with two Teflon nosepieces in the orthonasal condition or a flexible straw in the retronasal condition.
Olfactory stimuli in Experiment 2 consisted of reagent grade, non-reagent grade (Sigma-Aldrich; ≥ 99% and ≥ 99.5% purity for MSG and sucrose, respectively) and food grade (Ajinomoto Company and Imperial Sugar Company for MSG and sucrose, respectively) of 2.36 M MSG and 1.87 M sucrose diluted in double-distilled deionized water. Blank was double-distilled deionized water. The orthonasal apparatus setup was identical to the one used in Experiment 1.

To determine the olfactory thresholds for MSG and sucrose in Experiment 3, reagent grade MSG and sucrose were diluted in double-distilled deionized water to form 10 and 8 binary dilution steps from 2.8 M and 2.3 M, respectively. Phenylethyl alcohol (PEA) was diluted in propylene glycol from 4% v/v (0.33 M) to form 20 dilution steps. The blanks for the tastants and PEA were double-distilled deionized water and propylene glycol (PG), respectively. 10 ml of each serial dilution step and blank was presented in 280 ml glass bottle that was fitted with two Teflon nosepieces.

**Visual stimuli**

Visual stimuli in Experiment 4 consisted of a superimposed red/green MSG-rich food (steak) and sucrose-rich food (cake) image subtended a visual angle of 3.2° × 3.2°. Subjects viewed this composite steak/cake image through red/green anaglyph glasses, such that the steak image was presented to one eye and the cake image was presented to the other eye (Fig. 1). The color assignment to the image (green steak and red cake or vice versa) was counterbalanced across 30 subjects.
**Fig. 1. Visual stimuli used in Experiment 4.** Subjects viewed composite red steak/green cake or green steak/red cake image through red/green anaglyph glasses, so that the steak image was presented to one eye and the cake image was presented to the other eye.

*Procedure*

*Olfactory detection of basic tastes*

BMI was calculated as \( \text{weight (lb)} / [\text{height (in)}]^2 \) measured in the lab. To probe if the human nose can detect basic tastants, in Experiment 1, subjects performed 5 trials of triple-forced-choice discrimination tasks in which they orthonasally or retronasally smelled a set of three bottles (i.e., two containing the same tastants and one containing double-distilled deionized water) on each trial and selected the stimulus that smelled different. The retronasal condition was designed to exclude the potential confound by the interaction in the mouth. To investigate if the tastants have similar olfactory quality, 12 of them were randomly selected to perform similar tasks in a follow-up study to distinguish between MSG, sucrose and other tastants in the orthonasal condition. 30 sec inter-trial interval was used to prevent sensory fatigue. All subjects subsequently provided ratings of intensity, pleasantness and familiarity for the smell of each tastant and double-distilled deionized water on a 100-unit visual analogue scale (VAS), anchoring “not at all” and “extremely”. To rule out trigeminal irritation in subjects’ olfactory detection of tastants (Frasnelli et al., 2009; T. Hummel, Futschik, Frasnelli, & Hüttenbrink, 2003; Kleemann
et al., 2009; Kobal et al., 1989), subjects also performed a trigeminal task, in which they were presented with a tastant in one nostril and a blank control in the other nostril simultaneously and key pressed to indicate the side of the nostril that detected the smell. Each subject performed 12 trials (6 per nostril, 20 sec intertrial interval) of each tastant.

**Olfactory characteristics of basic tastants**

We were also curious to know the olfactory characteristics of basic tastants, so at the end of Experiment 1, all subjects were asked to describe what each tastant smelled like in the open-ended question and name the smell of tastants based on taste quality on a quintuple-forced-choice task (umami, sucrose, sour, salty and bitter) over 2 trials for each stimulus with 30 sec inter-trial interval.

**Olfactory detection of impure basic tastants**

To rule out possible confounds of impurity components in the olfactory stimuli, we used tastants in reagent grade. Moreover, subjects in Experiment 2 performed triple-forced-choice discrimination tasks, similar to Experiment 1, to discriminate the smell of MSG and sucrose in 3 different chemical grades from the blank control over a total of 60 trials (i.e., 10 trials per tastant). At the conclusion of the Experiment 2, they judged odor intensity, pleasantness and familiarity of each tastant on 100-unit VAS.

**Olfactory sensitivity to basic tastants across homeostatic states**

In Experiment 3, we investigated if olfactory sensing of basic tastants partakes in sensing nutrients and if its sensitivity fluctuates as a function of homeostatic states. Subjects performed olfactory threshold tasks orthonasally after 18 hours of fasting on one day (fasted session) and 30 min after consuming a 540-calorie meal (Big Mac) on another
day (fed session). The order of the two sessions was counterbalanced across subjects. The two sessions were conducted at the same time of the day, 1-2 days apart. Subjects brushed their teeth and rinsed their mouths with water prior to each testing session. They first completed hunger and alertness ratings on 100-unit VAS, anchoring “not at all” to “extremely”. The alertness rating was used to blind the main purpose of the study. The thresholds of MSG, sucrose and PEA were determined with triple-forced-choice ascending staircase design (Cain, Gent, Goodspeed, & Leonard, 1988; W. Li et al., 2007). The test started from the lowest concentration. On each trial, subjects were instructed to discern the smell of MSG, sucrose or PEA from two blank controls. When the incorrect answer was provided, a step higher concentration was used in the next trial. Otherwise, the same concentration was used on next trial until subjects achieved 5 consecutive hits. There was a 30 sec break between the trials. The order of test stimuli was randomized.

**Visual-olfactory sensing of the nutrients in basic tastants**

To study if olfactory detection of tastants facilitates visual search of nutrient, we conducted Experiment 4. At the beginning of the Experiment 4, subjects performed triple-forced-choice discrimination task, similar to Experiment 1, to discriminate MSG and sucrose separately from blank control over 2 trials. Then, subjects engaged in four 1-minute-long binocular rivalry trials, in which they viewed a composite red/green steak/cake image through red/green anaglyph glasses and pressed one of two keys whenever perceiving percept switched from steak to cake or cake to steak. At the same time, they were instructed to sample the smell continuously by inhaling through the nosepieces and exhaling through the mouth. Each of the two olfactory conditions (MSG and sucrose) was presented in two trials, making a total of 4 trials in random order with 2
min intertrial intervals. At the conclusion of Experiment 4, subjects rated odor intensity, pleasantness and familiarity of MSG and sucrose on 100-unit VAS.

Data analyses

For the data collected from Experiment 1, 2 and 4, one-sample t tests were performed to compare response accuracy (%) in discrimination, trigeminal, olfactory identification of taste solutions, and taste identification of taste solutions with chance levels at 33%, 50%, 20% and 16.67% respectively. Bonferroni adjustments were applied to the multiple comparisons.

Discrimination accuracy in Experiment 2 was further subjected to repeated measures of analysis of variance (ANOVA) with 3 chemical grades (reagent grade, non-reagent grade and food grade) as within-subject factor.

Intensity, pleasantness and familiarity ratings were separately entered into repeated measures ANOVA with 6 levels of olfactory stimulus (MSG, sucrose, citric acid, NaCl, quinine and water) in Experiment 1, 3 levels of chemical grade (reagent grade, non-reagent grade and food grade) in Experiment 2, and 2 levels of olfactory stimulus (MSG and sucrose) in Experiment 4. Any significant effects among olfactory stimuli were further analyzed by post hoc paired-samples t-test with Bonferroni adjustments.

The descriptions of olfactory sensation, acquired in Experiment 1, were categorized as food (e.g., plum, carrot), non-food (e.g., fabric, wood) or no smells (e.g., air, water). The frequency of description category (food, non-food and no smells) within a given olfactory stimulus was analyzed by 2-tailed chi-squared test. Post hoc analysis was applied to significant differences.
In Experiment 3, paired t-tests were carried out to compare hunger and alertness ratings; and MSG, sucrose and PEA olfactory thresholds across fasted and fed sessions.

In Experiment 4, the dominance time was calculated by averaging the time interval between pressing the two different keys, indicating the mean predominance duration \( (d) \) after switching to one percept and before switching to the other. Then, the mean predominance duration of steak image was divided by the sum of the mean predominance duration of steak and cake images, converting the mean predominance duration of steak image to proportion \( \text{prop}_{\text{steak}} = \frac{d_{\text{steak}}}{d_{\text{steak}} + d_{\text{cake}}} \), and vice versa \( \text{prop}_{\text{cake}} = \frac{d_{\text{cake}}}{d_{\text{cake}} + d_{\text{steak}}} \) or \( \text{prop}_{\text{cake}} = 1 - \text{prop}_{\text{steak}} \). The proportions of viewing a predominant steak image were significantly correlated between the two trials \( (r = 0.54, p < 0.001) \), so the mean proportion of predominant steak image was collapsed across two trials and entered into repeated measures ANOVA with olfactory stimulus (MSG and sucrose).

**Results and Discussion**

**Basic tastants confer olfactory sensation**

In Experiment 1, subjects orthonasally discriminated MSG and sucrose from double-distilled deionized water significantly above 33% chance level \( [t(29) = 4.80 \text{ and } 2.78 \text{ for MSG and sucrose, respectively, ps } < 0.05; \text{ Fig. 2A}] \). Yet, similar effects were not observed in other smells \( [t(29) = 1.99, 0.42 \text{ and } 0.31 \text{ for citric acid, NaCl and quinine, respectively, ps } > 0.05; \text{ Fig. 2A}] \). Moreover, subjects were unable to discriminate any of the smell when the smells were presented retronasally \( [t(29) = 2.31, 0.29, 2.27, 1.85 \text{ and } 0.46 \text{ for MSG, sucrose, citric acid, NaCl and quinine, respectively, ps } > 0.05; \text{ Fig. 2A}] \), suggesting that olfactory sensing of the taste solutions is not confounded by the
interaction of the smell in the mouth. Furthermore, the smell of MSG and sucrose was
discriminated significantly from the smell of each other and other taste solutions \([t(11) = 5.30, 4.07, 3.20 \text{ and } 4.09 \text{ for discriminating MSG from sucrose, citric acid, NaCl and quinine, respectively, } p < 0.05; \text{ Fig. 2B1; } t(11) = 13.42, 4.28, 3.30 \text{ and } 3.34 \text{ for discriminating sucrose from MSG, citric acid, NaCl and quinine, respectively, } p < 0.05; \text{ Fig. 2B2}], \) indicating that the smell of MSG and sucrose elicits qualitatively distinctive
olfactory sensations.

**Fig. 2. Olfactory discrimination of MSG, sucrose and other basic tastants.** (A) Subjects successfully discriminated MSG and sucrose from water orthonasally, but not retronasally. (B1) Subjects successfully discriminated MSG orthonasally from sucrose, citric acid, NaCl and quinine. (B2) Subjects successfully discriminated sucrose orthonasally from MSG, citric acid, NaCl and quinine. The asterisks, dash line and error bars represent \(p < 0.05, 33\% \text{ chance level and standard errors of the mean, respectively.} \)

In terms of intensity, pleasantness and familiarity, omnibus ANOVA revealed no
significant main effects of olfactory stimulus (MSG, sucrose, citric acid, NaCl, quinine
and water) \(F(4.53,131.24) = 1.85, p = 0.12\), Huynh-Feldt correction for intensity, \(F(5,145) = 0.34, p = 0.89\) for pleasantness and \(F(3.09,89.72) = 0.89, p = 0.46\), Greenhouse-Geisser correction for familiarity]. Trigeminal tests further confirmed that the smell of the tastants did not irritate the trigeminal nerve; none of the smell of the tastants can be localized reliably above the 50% chance level \([t(29) = 0.50, 0.87, 1.18, 0.19 \text{ and } 1.16 \text{ for MSG, sucrose, citric acid, NaCl and quinine, respectively, } p > 0.05]\).

After other possible confounds, including intensity, pleasantness, familiarity and trigeminal sensation, are also excluded, the argument that human detection of the smell of MSG and sucrose due to its unique olfactory quality is consolidated.

**Olfactory sensation of basic tastants defies conventional olfactory characterizations**

To be more specific to the idea of unique olfactory quality, subjects were asked to describe what each tastant smelled like. The descriptions of MSG, sucrose, citric acid and quinine differed significantly by category (i.e., food, non-food and no smells) \(\chi^2(2, N = 30) = 6.20, 7.80, 18.20 \text{ and } 7.80 \text{ for MSG, sucrose, citric acid and quinine, respectively, } p < 0.05; \text{ Fig. 3}\). The difference was marginally significant in NaCl \(\chi^2(2, N = 30) = 5.60, p = 0.06; \text{ Fig. 3}\). On the other hand, the description of water did not significantly vary across category \(\chi^2(2, N = 30) = 4.20, p = 0.12; \text{ Fig. 3}\). Post hoc analyses showed that the smells of taste solutions were mostly described as non-food related smell \(\chi^2(1, N = 150) = 38.88, p < 0.001; \text{ Fig. 3}\). Nevertheless, there was no consistent description of any given olfactory stimulus. For example, subjects described the smell of MSG solution as diverse as bread, cloth and book; and sucrose solution as rose, furniture and poker cards. In addition to the open-ended questions, we also probed the olfactory quality of these tastants by quintuple-forced-choice task. The identification
accuracy was not significantly beyond 20% chance level \( t(29) = 0.67, 0.30, 1.40, 0.27 \) and \( 0.00 \) for savory, sweet, sour, salty and bitter, respectively, \( p_s > 0.05 \). That is, the smell of MSG and sucrose is detectable by human noses, yet hard to be described by an existing odor category. Therefore, the smell of MSG and sucrose defies conventional olfactory categorization.

![Fig. 3](image-url)

**Fig. 3. The orthonasal smell of MSG, sucrose and other basic tastants are described as non-food related smells.** Subjects were asked to verbally describe what each basic tastant smelled like in open-ended questions. Except for water, the orthonasal smell of MSG, sucrose, citric acid, NaCl and quinine was primarily described as non-food related.

*Olfactory detection of tastants is not due to impurities in taste stimuli*

Past studies suspect the impurity of tastants contribute to the smell of tastants (Miller & Erickson, 1966; Mojet et al., 2005). Such concern is ruled out in animal studies where the solution intake, preference and lick latency did not significantly vary as the function of the purity level of the tastants (Rhinehart-doty et al., 1994; Zukerman et al., 2009). To rule out confound of impurity components, we introduced different chemical grades of MSG and sucrose to triple-forced-choice discrimination task in Experiment 2. The discrimination accuracy among tastants in different levels of purity versus double-distilled deionized water was significantly above 33% chance levels in both MSG \( t(9) = \)
4.13, 3.67 and 3.16 for reagent, non-reagent and food grades, respectively, ps < 0.05; Fig. 4] and sucrose \( t(9) = 4.98, 2.94, 5.79 \) for reagent, non-reagent and food grades, respectively, ps < 0.05; Fig. 4]. Moreover, the discrimination accuracy did not significantly differ by levels of purity \( F(2,18) = 0.26 \) and 2.16 for MSG and sucrose, respectively, ps > 0.05; Fig. 4]. In terms of odor intensity, pleasantness and familiarity the ratings, there are no significant main effects of level of purity in MSG \( F(2,18) = 0.04, p = 0.96, F(2,18) = 0.12, p = 0.89 \) and \( F(2,18) = 1.98, p = 0.17 \) for intensity, pleasantness and familiarity, respectively] and sucrose \( F(2,18) = 0.74, p = 0.49 \) for intensity, \( F(1.17,10.50) = 0.27, p = 0.65, \) Greenhouse-Geisser correction for pleasantness and \( F(2,18) = 0.12, p = 0.89 \) for familiarity).

In line with previous findings (Rhinehart-doty et al., 1994; Zukerman et al., 2009), Experiment 2 shows that the discrimination accuracy did not change systematically according to the level of purity, nor did odor intensity, pleasantness and familiarity ratings. In other words, the human nose detects the airborne chemicals released from MSG and sucrose per se, instead of their impure components.

**Fig. 4. Orthonasal discrimination of MSG and sucrose is independent of the level of chemical purity.** Subjects successfully discriminated MSG and sucrose from water, independent of chemical grades. Moreover, the performance did not significantly differ across chemical grades. The asterisks, dashed line and error bars represent \( p < 0.05, 33\% \) chance level and standard errors of the mean, respectively.
Olfactory sensitivity to tastes is modulated by hunger and satiety signals

Neural responses in orbitofrontal cortex has been found modulated by homeostatic states (Rolls, Sienkiewicz, & Yaxley, 1989) and the energy content of the food (Haase et al., 2009; Siep et al., 2009). To investigate if olfactory sensing of basic tastants partakes in sensing nutrients and if its sensitivity fluctuates is modulated by homeostatic states, subjects participated in fasted and fed sessions in Experiment 3. They were equally alert in fasted (M = 65.20, SEM = 5.69) and fed (M = 69.33, SEM = 5.31) sessions [t(14) = 0.75, p = 0.47], but they were significantly hungrier in fasted (M = 82.93, SEM = 3.57) than fed (M = 10.13, SEM = 3.30) sessions [t(14) = 13.47, p < 0.001], so the manipulation of homeostatic states was successful.

Mean olfactory thresholds (mean dilution steps) in fasted session were 5.68 (SEM = 0.61) for MSG, 2.60 (SEM = 0.40) for sucrose and 14.55 (SEM = 0.75) for non-food related smell PEA, and in fed session were 3.53 (SEM = 0.69) for MSG, 1.27 (SEM = 0.43) for sucrose and 14.27 (SEM = 0.73) for PEA. As illustrated in Fig. 5, olfactory sensitivities to MSG and sucrose were significantly higher (i.e., lower thresholds) in fasted session than fed session [t(14) = 2.99 and 3.57 for MSG and sucrose, respectively, ps < 0.05]. Olfactory thresholds to PEA remained the same across homeostatic states [t(14) = 0.41, p = 0.69].

Although subjects did not describe the smell of MSG and sucrose as food-related, olfactory sensitivity to the smell of MSG and sucrose increases when individuals feel hungry relative to satiated. Critically, such modulation effect is not observed in non-food related smell. Therefore, human noses can detect the nutritional values in the basic
tastants and olfactory sensitivity to the basic tastants is modulated by the homeostatic states.

Fig. 5. Homeostatic states modulate olfactory sensitivity to the smell of MSG and sucrose. Positive difference in threshold change represents increased threshold and decreased sensitivity from fasted to fed sessions. Olfactory sensitivities to MSG and sucrose solutions were significantly lower in fed than fasted sessions. The asterisks, error bars represent p < 0.05 and standard errors of the mean, respectively.

Olfactory sensing of taste facilitates visual perception of nutrient congruent food image

Subsequently, we explored the function of olfactory sensing nutrients in basic tastants. Subjects first successfully discriminated MSG and sucrose from the blank control above 33% chance level \[t(29) = 8.92 \text{ and } 9.33, \ p < 0.001 \text{ for MSG and sucrose, respectively},\] replicating finding in Experiment 1. The perceived intensity, pleasantness and familiarity did not significantly differ between MSG and sucrose \[F(1,29) = 1.78, 1.00 \text{ and } 2.39, \ p = 0.19, 0.33 \text{ and } 0.13 \text{ for intensity, pleasantness and familiarity, respectively}.\] In the binocular rivalry task, subjects significantly perceived predominant steak image longer when simultaneously smelling nutritional congruent (MSG) than incongruent (sucrose) tastants \[t(17) = 2.41, \ p = 0.03; \text{ Fig. 6},\] suggesting that the smell of tastants facilitates visual search for nutrient congruent food.
Fig. 6. The smell of MSG and sucrose modulate visual processing of nutrient congruent images. Subjects significantly perceived steak image longer in the presence of nutritional congruent (MSG) than the incongruent (sucrose) tastants. The asterisks and error bars represent p < 0.05 and standard errors of the mean, adjusted for individual differences, respectively.

In terms of perceived intensity, pleasantness and familiarity, there were neither significant main effects of substance (MSG and sucrose) [F(1,19) = .90, 3.03 and 0.52, p = 0.77, 0.10 and 0.48 for intensity, pleasantness and familiarity, respectively], modality (unimodal and bimodal) [F(1,19) = 1.31, 1.35 and 0.89, p = 0.27, 0.26 and 0.36 for intensity, pleasantness and familiarity, respectively], and sense (smell and taste) [F(1,19) = 0.13, 0.57 and 0.41, p = 0.72, 0.46 and 0.53 for intensity, pleasantness and familiarity, respectively], nor significant two-way [substance × modality: F(1,19) = 0.49, 0.02 and 1.02, p = 0.49, 0.90 and 0.33 for intensity, pleasantness and familiarity, respectively; substance × sense: F(1,19) = 3.50, 0.77 and 0.61, p = 0.08, 0.39 and 0.45 for intensity, pleasantness and familiarity, respectively; modality × sense: F(1,19) = 0.04 and 0.01, p = 0.84 and 0.91 for intensity and pleasantness, respectively] and three-way interactions [F(1,19) = 3.21, 3.08 and 2.35, p = 0.09, 0.10 and 0.14 for intensity, pleasantness and familiarity, respectively], except for modality by sense interaction in perceived familiarity [F(1,19) = 4.79, p = 0.04]. Post hoc tests with Bonferroni adjustments
revealed that subjects found smells, but not tastes, more familiar in unimodal than bimodal condition \([t(39) = 2.49, p = 0.001]\).

The olfactory description of subthreshold MSG and sucrose distributed equally in categories in both bimodal \(\chi^2(2, N = 20) = 1.9\) and \(1.3, p = 0.39\) and 0.52 for MSG and sucrose, respectively] and unimodal \(\chi^2(2, N = 20) = 1.9\) and 3.1, p = 0.39 and 0.21 for MSG and sucrose, respectively] conditions. Moreover, subjects could only identify subthreshold MSG and sucrose solutions at 16.67% chance level in both bimodal \([t(19) = 1.68\) and 0.84, p = 0.11 and 0.41 for MSG and sucrose, respectively] and unimodal \([t(19) = 1.27\) and 0.97, p = 0.22 and 0.35 for MSG and sucrose, respectively] conditions.

Evidence for olfactory sensing tastants is not new to animal literatures. By measuring behavioral responses, such as the latency of first lick on the solution and the amount of consumption of the solution, previous research robustly found animals detected sucrose and NaCl without tasting them, mediated by the surgery on olfactory system (Bell et al., 1979; Capaldi et al., 2004; Miller & Erickson, 1966; Privitera & Capaldi, 2006; Rhinehart-doty et al., 1994; Zukerman et al., 2009). Glutamate receptors were identified in animals’ olfactory organs as well (Abuin et al., 2011; Benton, Vannice, Gomez-Diaz, & Vosshall, 2009; Croset et al., 2010). Moreover, for MSG, sucrose and other tastants, their receptors are found in human nasal cavity (Finger & Kinnamon, 2011; Tizzano et al., 2011). As a result, biological evidence has already pinpointed the possibility of olfactory detection of tastants. Behavioral evidence from human subjects, however, is relatively scarce and equivocal (Henkin et al., 1962; Henkin & Powell, 1962; Hertz et al., 1975; Mojet et al., 2005). Our present study applied well-established olfactory testing methods, including triangular discrimination, intensity/pleasantness/familiarity judgment,
forced-choice identification and trigeminal tasks to all five basic tastants; and found subjects successfully detected MSG and sucrose solutions orthonasally. Subjects did not detect NaCl, but it was not unusual that animals have better olfactory sensitivity than humans (Can Güven & Laska, 2012; Laska & Seibt, 2002). Additionally, as compared with the water solubility in MSG (74g/100ml) and sucrose (200g/100ml), less NaCl (35.9g/100ml) can be dissolved in the same amount of water at room temperature. That is, the highest concentration of the solution we could prepare contained less NaCl as compared with MSG and sucrose solutions, so we speculated that its smell might less likely to be appreciated by the human nose.

After we have shown that the smell of each tastant confers unique olfactory quality, the next question is what do those smells smell like? In the open-ended questions, the smell of tastants was primarily described as non-food related smells. However, the descriptors vary from a wide range (e.g., fabric, book and rose) and hardly come into agreement. It has been known that humans are extremely good at discriminating smells but astonishingly poor at labeling and identifying smells (Yeshurun & Sobel, 2010). It is therefore not surprising that inconsistent descriptors were provided. We also found low accuracy in identifying the tastants based on their taste qualities, indicating that the smell of tastants did not carry the same perceptual qualities as its taste. In other words, like those taste receptors that have been found outside the oral area, the receptors in nasal cavity no longer signal the taste qualities, but instead regulate food ingestion and digestion (Finger & Kinnamon, 2011; Tizzano et al., 2011).

As the old saying goes, hunger is the best spice. In Experiment 3, after being abstained from food and beverages for 18 hours, subjects became more sensitive to the
smell of MSG and sucrose, but not to PEA, a non-food related smell. Such behavioral observations are supported by neural studies. It has been well documented that neural activities in olfactory system are more responsive and the levels of appetite-related neurotransmitters and hormones, whose receptors have been identified in olfactory circuits, are increased in hungry animals (Apelbaum et al., 2005; Breunig, Czesnik, et al., 2010; Breunig, Manzini, et al., 2010; Czesnik et al., 2007; Julliard et al., 2007; Pager et al., 1972; Pager, 1974; Root et al., 2011; Tong et al., 2011) and humans (Critchley & Rolls, 1996; O’Doherty et al., 2000; Tong et al., 2011). At the behavioral level, however, the change of olfactory sensitivity to food and non-food related smells across homeostatic states are controversial. In our study, although subjects described the smell of tastants was primarily non-food related, MSG and sucrose actually are known for carrying protein and energy, respectively (Naim, Ohara, Kare, & Levinson, 1991). Past studies also have commented that neural responses to sensory stimuli are determined by the interaction of food energy content and homeostatic states (Haase et al., 2009; Siep et al., 2009). In this regard, we suggest that olfactory modulation by homeostatic states is beyond individual’s knowledge and conscious awareness of the olfactory quality.

In Experiment 4, following the same paradigm in previous olfactory modulation on binocular rivalry study (Zhou et al., 2010), we found that a MSG-rich food – steak – was perceived more in a steak/cake rival image when subjects simultaneously sampled the smell of MSG solution. Similarly, a sucrose-rich food – cake – was perceived more in a steak/cake rival image when subjects simultaneously sampled the smell of sucrose solution.
Even though the true nature of stimulus is not accessible via verbal awareness, it affects the function of olfactory systems and behavioral responses as demonstrated in Experiment 3 and 4. Similar observations were made when human tears and sweat were served as the olfactory stimuli in which the chemosignals successfully mediate human perception of facial expressions, although subjects neither recognized the nature of stimuli, nor perceived the tears and sweat differently from controls based on intensity, pleasantness or familiarity (Gelstein et al., 2011; Zhou & Chen, 2009b). In a similar vein, although basic tastes are low in volatility, their olfactory properties defy conventional olfactory characterizations and modulate behavioral responses.

**Conclusion**

We show that humans detect the smell of basic tastants of MSG and sucrose, and that hunger enhances the sensitivity to the smells of MSG and sucrose but not that to the non-food smell. We also show that the smell of MSG and sucrose differentially biases subject’s visual perception of MSG- and sucrose-rich food in a binocular rivalry paradigm. We rule out interaction with the mouth, odorant intensity, pleasantness, familiarity, trigeminal sensation, or impurity components as alternative explanations in the olfactory detection of MSG and sucrose. Our findings suggest that olfactory detection of MSG and sucrose partakes in nutrient sensing, monitors homeostatic needs, and guides visual search for nutrient-rich food.
OVERALL SUMMARY

Taken together, the four studies shed new lights on understanding of the mechanisms, characteristics and functions of the human olfactory system.
REFERENCES


Percept Motor Skill, 92(3c), 1002–1008. doi:10.2466/pms.2001.92.3c.1002


