

fMRI Activation in Response to Odorants Orally Delivered in Aqueous Solutions

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Abstract

During food intake flavor perception results from simultaneous stimulation of the gustatory, olfactory and trigeminal systems. Olfactory stimulation occurs mainly through the retronasal pathway and the resulting perception is often interpreted as a taste perception, thus leading to the well-known sensory confusion between taste and olfaction. The present experiment was designed to study, with functional magnetic resonance imaging (fMRI), the cortical representation of olfactory perception in humans in response to retronasal stimulation by odorants delivered in aqueous solution. Psychophysical evaluation confirmed that the stimuli acted as pure olfactory stimuli through the retronasal pathway and did not present any taste component. Results showed activation in all brain regions previously described with neuroimaging techniques using olfactory stimulation with an odorized air flow. Piriform and orbitofrontal cortex were found activated as well as the hippocampal region, the amygdala, the insular lobe, the cingulate gyrus and the cerebellum. These results demonstrate the feasibility of efficiently stimulating the olfactory system in an fMRI scanner through the retronasal pathway with liquids delivered to the oral cavity. The presentation of olfactory stimuli in liquids to the mouth is a realistic model for the study of food-related flavor perception. This stimulation protocol furthermore allows presenting taste and olfactory stimuli separately or combined, thus allowing for direct comparisons between single modality representation, taste or olfaction, and representation of multi-modality mixtures.

Introduction

During food intake, flavor (Rozin, 1982) results from simultaneous stimulation of three main sensory systems: (i) taste, i.e. chemical stimulation of the taste buds of the tongue; (ii) olfaction, i.e. chemical stimulation of the olfactory epithelium, both through the orthonasal and the retronasal pathways; (iii) the trigeminal system, through chemical, thermal and tactile stimulation of the somato-sensory system, both on the tongue and on the nasal epithelium, lingual and nasal somatic stimulation.

Understanding the complex cross-modality interaction that constitutes the perception of flavor is a major prerequisite for the study of food-related behavior, its modifications and its disorders. Efficient functional brain imaging studies on flavor representation in the human cortex require a specific stimulation protocol designed to closely approximate natural flavor stimulation. The human olfactory system may be stimulated through two distinct pathways: the direct antero-posterior or orthonasal pathway, through the nostrils, and the retronasal pathway, involving the ascent of odorants through the posterior nares of the nasopharynx (Murphy *et al.*, 1977; Pierce and Halpern, 1996). The first pathway is naturally involved when we detect odors by passive or active smelling of stimuli presented in air to the nostrils. The retronasal pathway is much more likely to be

involved during the ingestion of food, when food molecules may efficiently pass from saliva to air due to mastication and heating in the mouth.

To date, studies of olfactory function with cerebral imaging techniques have used direct orthonasal stimulation with an odorized air flow (Kettenmann *et al.*, 1997; Yousem *et al.*, 1997; Zald and Pardo, 1997; Fulbright *et al.*, 1998; Sobel *et al.*, 1998a; Francis *et al.*, 1999; Lorig *et al.*, 1999; Kobal and Kettenmann, 2000; O'Doherty *et al.*, 2000) or stimulation with odorized cotton wands or swabs presented to the nostrils (Zatorre *et al.*, 1992; Koizuka *et al.*, 1994; Levy *et al.*, 1997; Small *et al.*, 1997). A few other studies used stimuli presented in the mouth which exhibited an olfactory component possibly stimulating the olfactory system through the retronasal pathway (Zald *et al.*, 1998; Gautier *et al.*, 1999). However, these studies could not dissociate the olfactory component of stimulation from the gustatory component associated with it and therefore did not allow investigation of the retronasal olfactory component alone. Thus, a study focusing specifically on the olfactory component related to flavor stimulation elicited in the oral cavity is lacking. It has been previously demonstrated psychophysically that presentation of odorant in aqueous solution in the mouth elicits retronasal olfaction

and permits study of the independent and relative contributions of olfaction and taste to flavor (Murphy *et al.*, 1977). The present experiment investigated, with functional magnetic resonance imaging (fMRI), the cortical representation of olfactory perception related to retronasal stimulation with odorants presented in aqueous solution in the mouth.

Materials and methods

Subjects and stimuli

Six young, healthy, right-handed adults participated in this study (three men and three women, aged 23–35 years) after giving informed consent. All had normal olfactory function, as assessed by odor threshold (Murphy *et al.*, 1990) and odor detection testing (Davidson and Murphy, 1997). The study was approved for the participation of human subjects by Institutional Review Boards at San Diego State University and the University of California San Diego. Each subject participated in one fMRI session of 1 hour or less, using a 1.5 T scanner (Siemens), with six functional runs of 5 min each with olfactory stimulation. During each run one stimulus was presented, alternating with water. Each subject was presented with two stimuli, one presented during the first three runs and the other during the last three runs. Order of stimuli was counterbalanced, so that no stimulus was presented systematically only first or only second. Stimuli included amyl acetate (0.02%), ethyl butyrate (0.02%) and citral (0.01%) and were chosen for their previous use in other experiments, presented either in air, i.e. amyl acetate (Murphy *et al.*, 2000; Wiser and Murphy, 2001), or in water, i.e. ethyl butyrate (Murphy *et al.*, 1977) and citral (Murphy and Cain, 1980). Stimuli were dissolved in distilled water and were presented to the subject's mouth through plastic tubes, as boluses of 50 μ l delivered every 3 s through automatic syringes. The subject put a soft plastic tube containing the ends of the tubes delivering water and stimuli in his/her mouth and was instructed to place it on the tip of the tongue, symmetrically, so that the liquids would flow on the whole tongue before being swallowed by the subject. The stimulation paradigm was composed of a reference period of 12 s with water followed by three 'ON'–'OFF' cycles, each with one 18 s ON period with the stimulus and one 75 s OFF period with water as rinsing solution. Both stimulus and water were delivered at the same flow rate and were equilibrated in temperature in order to avoid any systematic mechanical or thermal stimulation. Subjects were asked to concentrate on their perception in order to be able to give magnitude estimates and hedonic ratings for the stimuli in each run at the end of the fMRI session. All subjects were trained to swallow in a horizontal position prior to the scanning session in order to avoid motion artifacts. fMRI images were examined for evidence of significant motion.

Psychophysical experiments

Debriefing of the subjects after the fMRI session

Immediately after exiting the fMRI scanner subjects were asked to describe their perceptions during the functional runs. For each functional run subjects were asked to describe the stimulus, to decide if it was a taste, an odor or a combination of both, to report perceived intensities on a labeled magnitude scale from 0 to 100 (Green *et al.*, 1996) and to rate the pleasantness on a scale from –10 (very bad) to +10 (very good). Since each fMRI session began with acquisition of the structural parameters, subjects exited the scanner less than 40 min after the end of the first functional run, so that none of them reported having difficulty remembering the intensity and pleasantness of the stimuli over this period.

Perception profile recording

After the fMRI session all the subjects performed a series of psychophysical tests in the laboratory. Each subject participated in a simulated fMRI session and was presented with the same olfactory stimuli under simulated scanning conditions, i.e. the subject was lying on their back and was asked to swallow regularly without moving the head. During each simulated run the subject was instructed to continuously indicate the intensity of perception through the distance between the thumb and forefinger (finger span method) as he/she manipulated a linear potentiometer (Berglund *et al.*, 1978; Larson-Powers and Pangborn, 1978; Yamamoto *et al.*, 1985). The resulting perception profiles were digitized and stored (Figure 1). For each subject all three profiles corresponding to one and the same stimulus were averaged and used for data processing of fMRI images (Van de Moortele *et al.*, 1997).

Magnitude estimates

Perceived intensity was also assessed for the odors presented in the scanner and for water. Stimuli were presented in the same manner and in the same amount delivered in the scanner during one ON period (18 s = 6 $\times$ 50 μ l). Subjects reported perceived intensities using a labeled magnitude scale from 0 to 100 (Green *et al.*, 1996) for each stimulus under two conditions: with or without retronasal olfaction, i.e. with nostrils open or pinch-closed (Murphy *et al.*, 1977). Stimuli were presented twice in randomized order for each condition.

fMRI scanning and data processing

Each imaging session began with the acquisition of high resolution anatomical images to allow accurate localization of activations (MPRAGE, 180 sagittal slices, FOV 256, 1 mm thick, resolution 1 $\times$ 1 $\times$ 1 mm³, TR 11.4 s, TE 4.4 ms, flip angle 10°). Then six functional runs of 5 min were performed with an echo planar sequence to acquire functional images (32 sagittal slices, FOV 256, resolution 4 $\times$ 4 $\times$ 4 mm³, TR 4 s, TE 40 ms, flip angle 90°).

Functional data were processed with AFNI (analysis of functional neuroimages) software (Cox, 1996). Each functional run (echo planar image) was composed of 77 temporal volumes (number of repetitions) of 32 sagittal slices each. The first three volumes corresponding to the stabilization period of the magnetic signal were not considered for further analysis. Each run's temporal series were temporally smoothed and were re-aligned to correct for small movements. Resulting motion correction equations indicated that movement did not exceed 2 mm in translation or rotation for any subject. Thus no run needed to be discarded because of movement. For each subject all three runs corresponding to one and the same stimulus were averaged. Low signal intensity voxels corresponding to voxels located outside the brain were discarded from the functional images (echo planar time series) by a clipping function.

Analysis of individual runs

Each averaged run was correlated with a template based on the *post hoc* averaged perception profile given by each subject for each stimulus. The template was shifted in time 2 s before and 3 s after, in 1 s shifts, to account for delays in time acquisition between the first and the last slice of each temporal volume. Voxels with a correlation coefficient exceeding a threshold of 0.42 ($P = 0.001$) and belonging to clusters of at least two voxels were considered as activated. These parameters were chosen on the basis of 10 000 Monte Carlo simulations processed with the AlphaSim program (Ward, 1997). The program estimates the probability of occurrence of clusters composed of voxels with a specific P value (i.e. 0.001), separated by no more than 1 voxel width (i.e. 4 mm, meaning that activated voxels in the same cluster had one complete side in common), for images spatially blurred with a 4 mm kernel (FWHM) Gaussian filtering. The analysis indicated that with the parameters of the present study <5% of clusters would be activated by chance in the complete explored brain volume. Images were then normalized to fit the Talairach coordinate reference system (Talairach and Tournoux, 1993) using the AFNI algorithm. Activated areas were identified using Talairach coordinates and human brain atlases (Talairach and Tournoux, 1993; Mai *et al.*, 1997).

Group analysis

Group analysis was performed on 12 data sets (one average run for each of two stimuli for each of the six subjects) transformed to Talairach space. A one sample t -test was calculated on the percent change observed at each voxel. Voxels presenting $P < 0.0125$ and belonging to clusters of at least two voxels were considered as activated. Less stringent statistical thresholds were chosen at the group level than at the individual level in order to limit the loss of power due to inter-individual differences in activation localization.

Results

Psychophysical measures

Magnitude estimates and hedonic ratings recorded at the conclusion of the fMRI scanning session provided information regarding the intensity and quality of the perception and the level of attention of the subjects. All the subjects reported having focused their attention on the stimuli.

Post hoc magnitude estimates with nose open or closed indicated the following.

- Perceived intensities for ethyl butyrate were significantly higher when presented with the nose open ($M = 20.5 \pm 10.0$) than with the nose closed ($M = 2.9 \pm 4.2$, $t(18) = 4.53$, $P < 0.001$). The same result was obtained for citral ($M = 8.6 \pm 6.2$ with the nose open, $M = 0.6 \pm 0.9$, with the nose closed, $t(16) = 3.18$, $P < 0.01$) and for amyl acetate ($M = 15.5 \pm 6.7$ with the nose open, $M = 3.9 \pm 4.9$ with the nose closed, $t(16) = 4.6$, $P < 0.001$).
- In contrast, as expected, perceived intensities for water presented with the nose open ($M = 1.0 \pm 2.1$) or the nose closed ($M = 1.0 \pm 1.2$) were not significantly different [$t(18) = 0.00$, NS].
- Perceived intensities for ethyl butyrate, citral and amyl acetate with the nose closed were not significantly different from perceived intensities for water with the nose closed (comparison with ethyl butyrate, $t(18) = 1.25$, NS; comparison with citral, $t(16) = 0.35$, NS; comparison with amyl acetate, $t(16) = 1.00$, NS).

These measurements confirmed that the stimuli used in the fMRI experiment actually acted as olfactory stimuli perceived through the retronasal pathway rather than as gustatory stimuli. Thus description of these stimuli as 'tastes' by some of the subjects was a true sensory confusion, based on the presentation of an olfactory stimulus in a liquid form to the mouth, where taste sensation is expected by the subject (Murphy *et al.*, 1977).

Perception profiles recorded for each subject during simulated fMRI experiments, illustrated in Figure 1, provided information regarding the accuracy and reproducibility of the subject's perception with the stimulation paradigm used. They also suggested that the stimulation paradigm efficiently limited adaptation, since the magnitude estimate represented by the height of the profile did not significantly decrease either during one ON period or across ON periods. The perception profiles were then used as templates for extracting brain activations in the fMRI images.

fMRI results

Group analysis

The group analysis identified the main regions of interest activated for the six subjects (Figure 2). Activation was

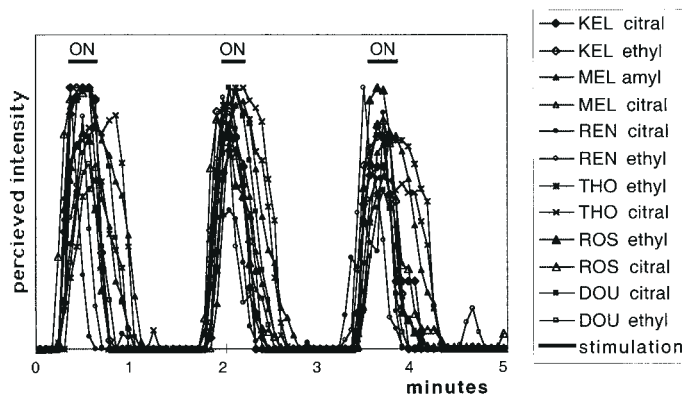


Figure 1 Average perception profiles for each subject and each stimulus. Perception profiles were collected during simulated fMRI experiments performed after the scanning session. The subjects used a linear potentiometer to continuously indicate the magnitude of their perception by the distance between their thumb and forefinger (finger span method). ON indicates when the stimulus was actually delivered to the subject. Three profiles were recorded from each subject for each stimulus and were then averaged. The resulting perception profiles were used as templates for extracting brain activations in the functional MR images. Individual subjects were coded by three capital letters. Ethyl, ethyl butyrate; Amyl, amyl acetate.

localized in primary olfactory areas, especially in right piriform cortex, and in other olfactory areas, including left and right orbitofrontal cortex, the left hippocampus, the right parahippocampal gyrus and the right amygdala. Other areas were found bilaterally activated, including the insula, the temporal operculum and the rolandic operculum (base of pre- and post-central gyri, the cingulate gyrus and the cerebellum (Table 1).

In the insular lobe different sub-regions were found activated (see Figure 2). The ventral part of the insula was found activated in both the right and left hemispheres (respectively 2 and 0 mm below the AC–PC line in the left and right hemispheres). Two additional foci of activation could be identified in the right dorsal insula (16 and 18 mm above the AC–PC line, respectively). One focus was localized in the medial portion (3 mm anterior to AC) and the other in the posterior portion (12 mm posterior to AC).

Individual analysis

The individual analysis was performed on each of the two runs performed by the six subjects with more stringent thresholds than the group analysis. With these parameters three runs exhibited a very low level of activation overall (KEL ethyl, REN ethyl, REN citral). This low level of activation was not systematically associated with low magnitude estimates for the stimuli and did not seem to correspond to a low level of attention. In these runs no or very little activation was detected in areas of interest detected in the group analysis, but for all other runs activation was detected in some or all of them (Figure 3 and Table 2).

Concerning the main regions of interest identified with the group analysis, individual runs revealed activation in the right amygdala for three subjects and in either left or right piriform cortex for three subjects, in the entorhinal and parahippocampal gyrus for four subjects, in orbitofrontal cortex for all six subjects, in the hippocampus for three subjects and in the insula for five subjects.

Interestingly, individual results did not completely duplicate group analysis findings and provided additional information about localization and lateralization of activations. Although group analysis detected activation in right piriform cortex only, individual analysis revealed activation in piriform cortex in either the right hemisphere or the left hemisphere. Individual analysis also revealed larger activations and more foci in left orbitofrontal cortex than in the right one, whereas group analysis showed a globally bilateral activation. A similar observation could be made for the insula, which appeared more activated in the right hemisphere at the group level but more often activated in the left hemisphere in the individual analysis (Table 2).

Discussion

The goal of the current study was to determine whether retronasal stimulation with odorant in aqueous solution in the mouth was capable of activating areas of the human brain that had been shown in previous studies to be activated by stimulation with odorant in the air stream in the anterior nares. The results clearly confirmed that retronasal olfactory stimulation activates cortical areas that correspond to those described in previous neuroimaging studies of olfaction (see Figure 4).

The results of the present study have been analyzed with two complementary analysis strategies, the first emphasizing data from individual subjects and thus capitalizing on the strength of fMRI in precise localization of cortical activation and the second emphasizing the group trends and thus allowing for comparisons with previous studies with positron emission tomography (PET).

The group analysis identified regions involved in the cortical representation of retronasal olfactory perception for all six subjects. These regions included piriform cortex, the parahippocampal gyrus (posterior part of entorhinal cortex), orbitofrontal cortex, the hippocampus, the amygdala, the insula, the cingulate gyrus and the cerebellum (Figure 4). Piriform cortex, the amygdala and lateral entorhinal cortex have been shown in the primate to receive direct projections from the olfactory bulb and may thus be considered as parts of the primary olfactory cortex (Carmichael *et al.*, 1994). Further projections have been described from entorhinal cortex to the hippocampus and from piriform cortex to entorhinal cortex and to orbitofrontal cortex, which also receives projections from the mediodorsal thalamic nucleus. Previous neuroimaging studies of olfactory function have only rarely reported

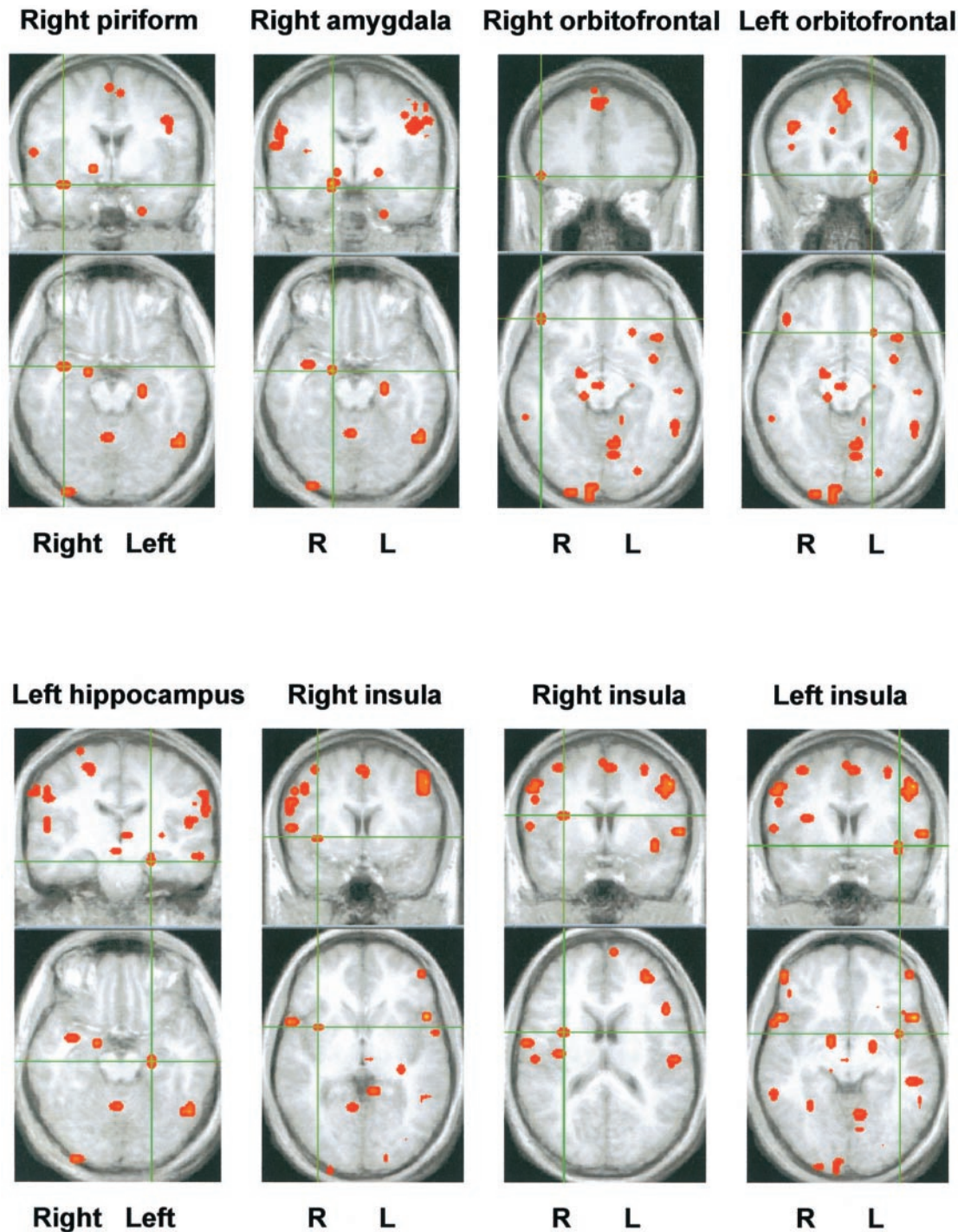


Figure 2 Cortical activation for the group of six subjects in response to retronasal olfactory stimulation. Individual datasets were transformed to Talairach space using an AFNI algorithm. A one-sample t -test was calculated on the percent change observed at each voxel. Voxels presenting $P < 0.0125$ and belonging to clusters of at least two voxels were considered as activated. Activation maps were superimposed on high resolution anatomical images to allow accurate localization. The left side of the brain is presented on the right side of the image according to radiological convention. Crosshairs indicate the location of the observed activation foci in both the coronal (upper view) and horizontal (lower view) planes. Examples of activation are shown in right piriform cortex, the right amygdala, right and left orbitofrontal cortex, the left hippocampus and the right and left insula.

activation in piriform cortex (Zatorre *et al.*, 1992; Small *et al.*, 1997; Dade *et al.*, 1998; Savic *et al.*, 2000; Sobel *et al.*, 2000), the amygdala, the entorhinal or parahippocampal

gyrus and the hippocampus (Small *et al.*, 1997; Zald and Pardo, 1997, 2000b; Sobel *et al.*, 2000). In contrast, the majority of previous neuroimaging studies on olfaction have

Table 1 Activations detected with the group analysis in response to retronasal olfactory stimulation

ROI	Hem.	Vox.	Talairach coordinates		
			X (mean $\pm$ SEM)	Y (mean $\pm$ SEM)	Z (mean $\pm$ SEM)
Piriform cortex	R	2	35.9	-1	-16
Amygdala	R	4	16.9	-7.3	-13
Parahippocampal g.	R	2	30	-51	-4
Hippocampus	L	3	-22	-18.4	-14.9
Orbitofrontal cortex	L	5	-31.6 $\pm$ 6.8	21 $\pm$ 1.4	-10 $\pm$ 0.1
Orbitofrontal cortex	R	4	48 $\pm$ 2.8	39.3 $\pm$ 8.9	-6 $\pm$ 2.8
Insula	L	2	-38	3	-6.4
Insula	R	10	34.7 $\pm$ 2.2	-0.6 $\pm$ 9.9	10.1 $\pm$ 8.8
Cingulate g.	R/L	24	2	23	43
Cerebellum	L	49	-25 $\pm$ 16.7	-61.8 $\pm$ 8.9	-27.4 $\pm$ 20.7
Cerebellum	R	21	21.5 $\pm$ 18.1	-61.5 $\pm$ 16.4	-27.4 $\pm$ 8
Temporal pole/op.	L	4	-49.2	15	-1.6
Temporal pole/op.	R	5	54.6 $\pm$ 3.2	13 $\pm$ 2.8	-2.7 $\pm$ 1.8
Frontal op.	R	5	37.1 $\pm$ 6.9	19.4 $\pm$ 0.6	17.2 $\pm$ 9.6
Rolandic op.	L	7	-56.7 $\pm$ 1.4	-12.3 $\pm$ 17.2	16.9 $\pm$ 11.2
Rolandic op.	R	2	60.2	-5	16
Postcentral g.	L	38	-41.6 $\pm$ 16.3	-22.9 $\pm$ 9.8	45.5 $\pm$ 17.4
Postcentral g.	R	22	38.3 $\pm$ 18.7	-22.4 $\pm$ 8.2	52 $\pm$ 16.4
Precentral g.	L	21	-52.6 $\pm$ 4.5	-4 $\pm$ 8	40.5 $\pm$ 5.4
Precentral g.	R	14	52.1 $\pm$ 4.5	7 $\pm$ 3	33.3 $\pm$ 5.5
Medial frontal g.	L	30	-32.8 $\pm$ 8.7	30 $\pm$ 18.7	25.2 $\pm$ 14.9
Medial frontal g.	R	10	36.8 $\pm$ 4.9	14.7 $\pm$ 24.3	37 $\pm$ 19.1
Angular gyrus	L	6	-45.3 $\pm$ 11	-58.3 $\pm$ 12.9	37.4 $\pm$ 11.5
Supramarginal g.	L	20	-42.9 $\pm$ 9.6	-49.2 $\pm$ 12.6	37.8 $\pm$ 5
Supramarginal g.	R	19	40.5 $\pm$ 8.4	-51.2 $\pm$ 15.6	37.4 $\pm$ 4
Superior frontal g.	L	25	-5.3	12.7	54.8
Superior frontal g.	R	24	2	22.6	43.3
Temporal lobe	L	15	-53.8 $\pm$ 4.7	-41.9 $\pm$ 15.3	1 $\pm$ 14.3
Temporal lobe	R	3	58	-42.4	-5.3
Occipital gyri	L	10	-23 $\pm$ 6.3	-82.2 $\pm$ 12.5	-13.7 $\pm$ 10.9
Occipital gyri	R	24	21 $\pm$ 8.9	-97.5 $\pm$ 4.4	-4.1 $\pm$ 12.3
Total voxels table		430			
Total voxels brain		513			

Talairach coordinates were expressed according to Talairach and Tournoux conventions (X, left to right; Y, posterior to anterior; Z, inferior to superior). The overall number of voxels in the areas displayed (430 voxels) represents 84% of the total number of activated voxels in the whole brain volume (513 voxels). ROI, region of interest; Hem., hemisphere; vox, number of activated voxels; g., gyrus; op., operculum; R, right; L, left.

reported robust activation in orbitofrontal cortex (Zatorre *et al.*, 1992; Levy *et al.*, 1997; Small *et al.*, 1997; Yousem *et al.*, 1997; Fulbright *et al.*, 1998; Sobel *et al.*, 1998a, 2000; Francis *et al.*, 1999; Kobal and Kettenmann, 1999, 2000; O'Doherty *et al.*, 2000; Savic *et al.*, 2000; Zald and Pardo, 2000b; Zatorre and Jones-Gotman, 2000). Other areas have also been consistently found to be activated in response to olfactory stimulation with functional neuroimaging techniques, including the insula, the anterior cingulate gyrus and the cerebellum (Zatorre *et al.*, 1992; Levy *et al.*, 1997; Small *et al.*, 1997; Yousem *et al.*, 1997; Fulbright *et al.*, 1998; Sobel *et al.*, 1998b; Francis *et al.*, 1999; Kobal and Kettenmann, 2000; O'Doherty *et al.*, 2000; Savic *et al.*, 2000; Zald and Pardo, 2000b).

The group analysis performed in the present study detected activation related to retronasal olfactory perception in the principal cortical areas of the olfactory system and in other areas that have been consistently reported as activated in humans in response to orthonasal olfactory stimulation in previous functional neuroimaging experiments. Some of these activation areas warrant more discussion and will thus be considered in detail.

Activation in piriform cortex

Piriform cortex is a small structure in humans and its proximity to the insular lobe may make identification of activations in this area difficult, even with the good spatial resolution of fMRI. However, when activation was found in

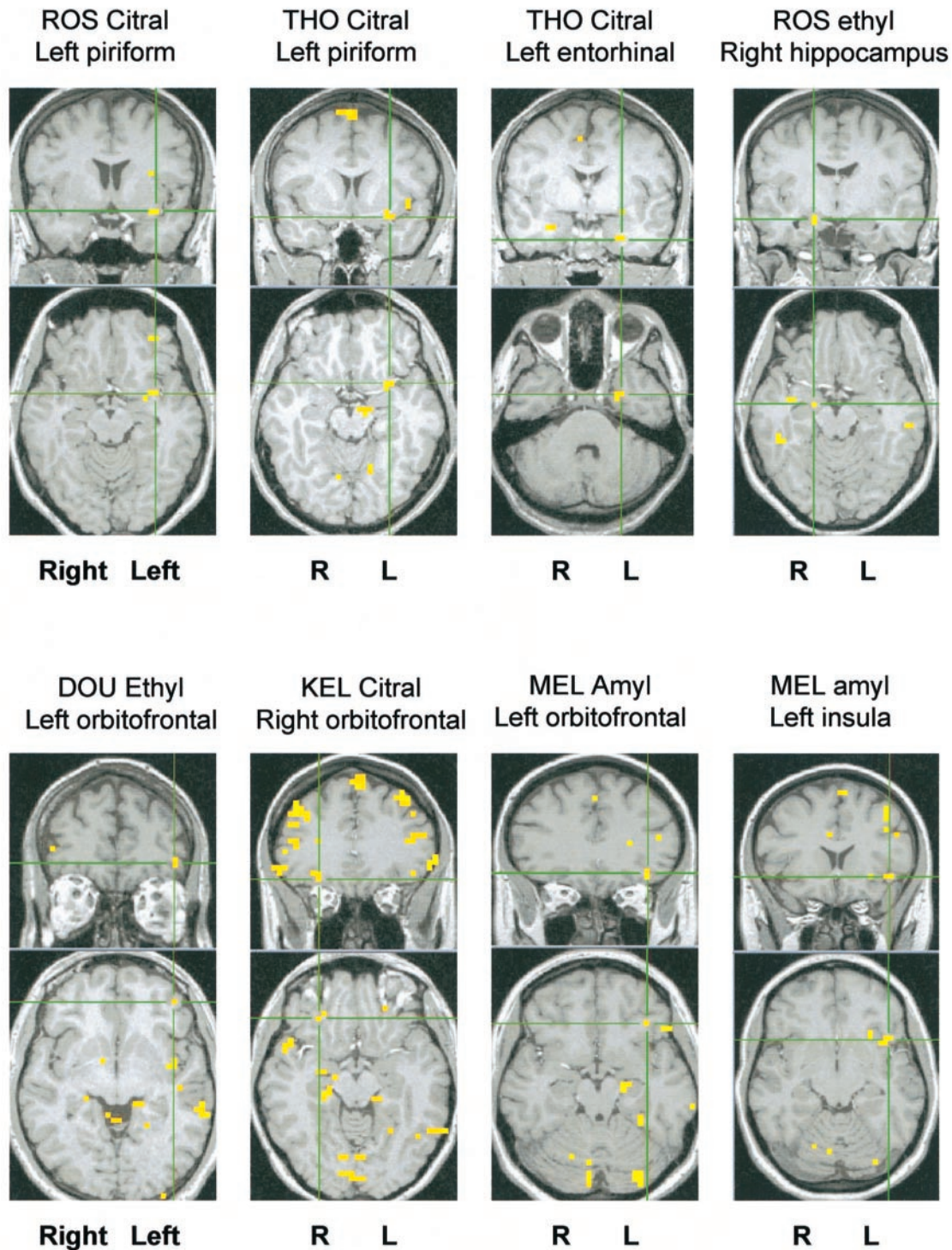


Figure 3 Activations in response to retronasal olfactory stimulation for individual runs. Activations were detected by correlation with the perception profiles given by each subject for each stimulus. Voxels with a correlation coefficient exceeding a threshold of 0.42 ($P = 0.001$) and belonging to clusters of at least two voxels were considered as activated. According to Monte Carlo simulations [AlphaSim (Ward, 1997)] <5% of clusters would be activated by chance in the complete explored brain volume with these parameters. Images were normalized to fit the Talairach coordinates reference system using the AFNI algorithm. Activation maps were superimposed on high resolution anatomical images to allow accurate localization. The left side of the brain is presented on the right side of the image according to radiological convention. Crosshairs indicate the location of the observed activation foci in both the coronal (upper view) and horizontal (lower view) planes. Subjects were coded by three capital letters. Ethyl, ethyl butyrate; Amyl, amyl acetate. Examples of activation are shown in left piriform cortex, left entorhinal cortex, right hippocampus, left and right orbitofrontal cortex and left insula.

Table 2 Activations detected in individual runs in response to retronasal olfactory stimulation

Brain region	Hem.	Stimulus/subject												n
		Amyl/ MEL	Citral/ MEL	Citral/ THO	Ethyl/ THO	Ethyl/ KEL	Citral/ KEL	Ethyl/ REN	Citral/ REN	Citral/ DOU	Ethyl/ DOU	Ethyl/ ROS	Citral/ ROS	
Pir	L			8									4	3
	R									2		2		
Ent/p.hip	L	6		4	2		11				6			4
	R	3			4									
Amyg	L													3
	R			2			15					2		
OBF	L	4	6	2			25				2	2	6	6
	R		2	2			8		3			2		
Insula	L	6	2	4	12		29			2	5		4	5
	R		5		2		27				6		7	
Hipp	L	7	4								2	2		3
	R	4	2				15				9	2		
Total		168	671	187	170	12	1918	40	45	113	394	130	149	6

The numbers of activated voxels found in major areas of interest are presented for each run corresponding to one stimulus for one subject. The last row shows the total number of voxels activated in the whole brain for each run. The last column presents the overall number of subjects exhibiting activation in each region of interest. Pir, piriform cortex; ent/p. hip, entorhinal cortex/parahippocampal gyrus; Amyg, amygdala; OBF, orbitofrontal cortex; Hipp, hippocampus; R, right; L, left; Amyl, amyl acetate; Ethyl, ethyl butyrate. Subjects were coded by three capital letters.

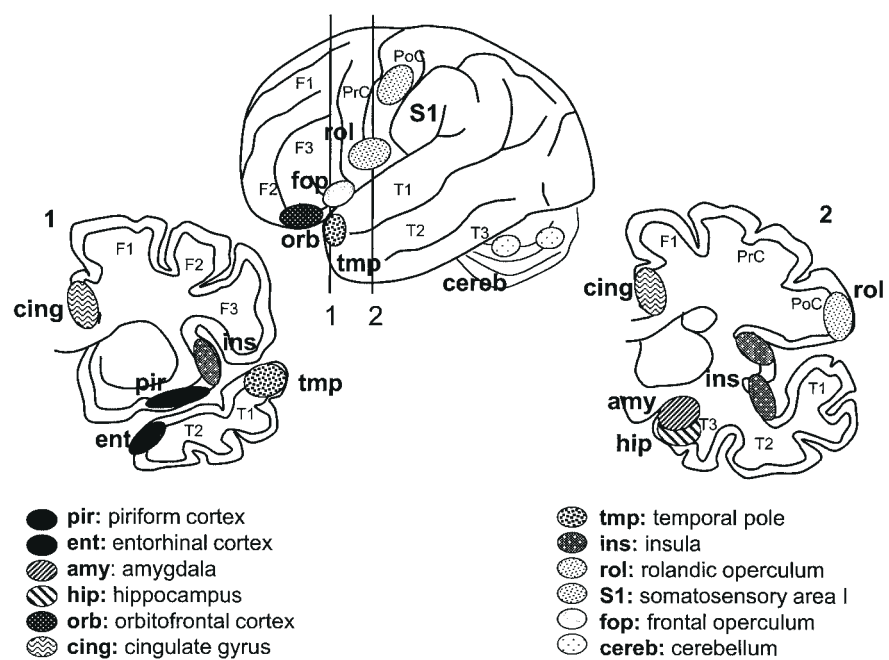


Figure 4 Schematic overview of principal areas found activated in response to retronasal olfactory stimulation with aqueous solutions delivered to the mouth. Activation was observed in all regions previously found activated by orthonasal stimulation with odorized air, including piriform cortex, entorhinal/parahippocampal gyrus, amygdala, hippocampus, orbitofrontal cortex, cingulate gyrus, temporal pole/operculum, insula, postcentral gyrus or somatosensory area I (S1) and cerebellum. Other areas were also found activated, in particular the rolandic operculum.

this region in the subjects included in the present study it was clearly dissociated from activation foci localized in other parts of the insular lobe. Although it is impossible to completely rule out the possibility that activations found in

this region comprised some parts of the ventral agaranular insula, these activations clearly included piriform cortex. Thus, activation was detected in the present study in the piriform area, both at the group level (Table 1) and at the

individual level (Table 2), whereas only few other neuroimaging studies on olfaction have reported activation in this region (Zatorre *et al.*, 1992; Small *et al.*, 1997; Dade *et al.*, 1998; Savic *et al.*, 2000; Sobel *et al.*, 2000).

Several authors have proposed different reasons for the inconsistency of activation of primary olfactory structures with neuroimaging techniques. In fMRI studies functional imaging using short acquisition times produces signal loss due to inhomogeneities in inferior frontal and lateral temporal areas, located near to air–tissue interfaces (Yang *et al.*, 1997; Frahm *et al.*, 1988), that may reduce the detectability of activations located in primary olfactory structures. However, as noted by Zald and Pardo, this factor alone cannot explain difficulties in detecting piriform activation with both fMRI and PET, since PET is not prone to the same artifacts (Zald and Pardo, 2000b).

Some authors suggested that piriform cortex would be activated by sniffing, so that activation during both odorant and no-odorant conditions would be canceled out by image subtraction methods, thus leading to a lack of robust activation in primary olfactory structures (Sobel *et al.*, 2000; Zald and Pardo, 2000b). In the present study we may note that sniffing and olfactory perception were completely dissociated, since olfactory perception occurred through the retronasal pathway, which is not likely to be involved during sniffing but rather during expiring or after swallowing. Thus this method of stimulation may have facilitated the detection of activation in primary olfactory cortex.

Adaptation and/or habituation effects occurring over the long stimulation periods necessitated by the fMRI and PET techniques utilized to date may also lead to difficulties in detecting activation in primary olfactory structures. A continuous olfactory stimulation over durations of up to 60 or 90 s may indeed cause a fading of the perception, up to 30% of the magnitude of the original perception (Ekman *et al.*, 1967), and a decrease in activity in piriform cortex, possibly down to baseline (Wilson, 1998). In the present study stimulation periods (ON) with odorant were short (18 s) and were followed by long-lasting rinsing phases (75 s) intended to limit adaptation over runs. The delivery of small amounts of stimulus (50 μ l) every 3 s also limited adaptation during each ON period (McBurney, 1976). Consequently, the present stimulation paradigm actually limited adaptation, as indicated by perception profiles (see Figure 1). Moreover, the template used to retrieve brain activations was based on the actual perception of the subject rather than on the stimulation (Van de Moortele *et al.*, 1997). This template particularly accounts for the slow return to baseline of the perception after the end of the stimulation and thus maximizes the extraction of activations related to the perception (see Figure 1). Limiting adaptation and the use of a template to extract activations based on actual perception may have facilitated the detection of activation in primary olfactory structures in the present study, in contrast to previous neuroimaging studies.

Finally, more cognitive parameters, such as novelty or familiarity of the odor, may also influence the degree of activation in piriform cortex or other primary olfactory structures. Dade *et al.* detected piriform activity with PET during odor recognition but not during odor encoding (Dade *et al.*, 1998). In the present study subjects were asked to focus their attention on the quality and intensity of the stimulus, as they would be asked to identify and provide magnitude estimates and hedonic ratings for each stimulus at the end of the imaging session. It is possible that this specific task produced activation in piriform cortex whereas other tasks did not.

Activation in orbitofrontal cortex

In the present study activation was detected in orbitofrontal cortex in both the left and right hemispheres (Table 1) and every subject exhibited activation in orbitofrontal cortex in response to at least one stimulus (Table 2). The individual analysis revealed that orbitofrontal cortex activations were found more often and were larger in the left hemisphere than in the right hemisphere.

Orbitofrontal cortex is a large heterogeneous region and the different activation foci found either in the left or the right hemisphere in different parts of orbitofrontal cortex may actually be involved in different aspects of the processing of olfactory information. Nonetheless, the predominant left orbitofrontal activation at the individual level contrasts with a number of previous PET studies describing a predominant activation of orbitofrontal cortex in the right hemisphere (Zatorre *et al.*, 1992, 2000; Small *et al.*, 1997; Yousem *et al.*, 1997; Sobel *et al.*, 1998a; Francis *et al.*, 1999; Royet *et al.*, 1999; Savic *et al.*, 2000). The dominance of right orbitofrontal activation has often been interpreted as a general superiority of the right hemisphere for olfactory processing, consistent with some clinical observations that show greater olfactory deficits due to right orbitofrontal lesions (Zatorre and Jones-Gotman, 1991; Jones-Gotman and Zatorre, 1993) and better discrimination performance when odors are presented to the right nostril (Zatorre and Jones-Gotman, 1990).

However, other studies tend to moderate this interpretation. Zald and Pardo described left orbitofrontal activation in response to exposure to a highly aversive odorant (Zald and Pardo, 1997). They reported that this left activation could also be observed for pleasant odorants (Zald and Pardo, 2000b). A recent fMRI study described activation in orbitofrontal cortex in both the right and left hemispheres of individual subjects in response to banana odor (O'Doherty *et al.*, 2000) and another fMRI study reported left orbitofrontal activation in response to tea and vanillin odors (Bowtell *et al.*, 2000). Royet *et al.* reported that emotionally valenced olfactory, visual and auditory stimuli, regardless of the sensory modality, preferentially activated the left hemisphere, including left orbitofrontal cortex (Royet *et al.*, 2000). Thus, the relationship between lateral-

ization of orbitofrontal activation in olfactory processing and the hedonic value of odor perceptions still remains unclear. The predominant left orbitofrontal activation described in the present study at the individual level and in other fMRI studies on olfaction (Bowtell *et al.*, 2000; O'Doherty *et al.*, 2000) may suggest that activation of the left orbitofrontal cortex would be more easily detected with fMRI individual analyses than with PET group analyses that average results from different individuals. Important inter-individual variability in localization may indeed reduce the detectability of orbitofrontal activation foci in the left hemisphere with group analyses but less with individual analyses.

Another stream of data also suggests that the differences in lateralization of orbitofrontal activation among different studies could be related to different cognitive tasks performed by the subject or to different characteristics of the stimulus. Zatorre *et al.* described right orbitofrontal activation with PET for both pleasantness and intensity judgements (Zatorre *et al.*, 2000). Royet *et al.* showed with PET that right orbitofrontal cortex would be more activated for familiarity judgements whereas left orbitofrontal cortex would be significantly activated during hedonic judgements but not during odor detection or edibility judgements (Royet *et al.*, 2000). In the present study magnitude estimate and hedonic rating tasks may have emphasized left orbitofrontal cortex activation whereas other cognitive tasks may not have. Taken together, these results suggest that different cognitive tasks may affect the lateralization of cerebral processing associated with olfactory perception.

Activation in the insula

Portions of the insula-claustrum receive direct projections from the olfactory system (Carmichael *et al.*, 1994) and insular activation in response to odorants delivered in air has been reported in previous neuroimaging studies (Zatorre *et al.*, 1992; Small *et al.*, 1997; Fulbright *et al.*, 1998; Zald *et al.*, 1998; Francis *et al.*, 1999; O'Doherty *et al.*, 2000; Savic *et al.*, 2000; Zald and Pardo, 2000b). Furthermore, the insula also receives direct projections from the gustatory system (Norgren, 1990) and robust activation in response to taste stimulation has been observed with neuroimaging techniques (Kinomura *et al.*, 1994; Kobayakawa *et al.*, 1996; Murayama *et al.*, 1996; Cerf *et al.*, 1998; Faurion *et al.*, 1999; Small *et al.*, 1999).

In the present study the group analysis revealed three different foci of activation in the right insula and one in the left insula. The left insular focus and one right insular focus were localized in the ventral part of the insular lobe, corresponding to the region described in non-human primates as receiving projections from the primary olfactory cortex (Shipley and Geinisman, 1984). The identification by magnetoencephalography (MEG) of a late component (434 ms) in response to olfactory stimulation in the left insula suggests the notion of secondary or tertiary olfactory

projection in the ventral insula (Kettenmann *et al.*, 1997). Furthermore, a previous fMRI study on taste perception has reported that the ventral insula was found predominantly unilaterally and symmetrically activated in left-handed and right-handed subjects, in contrast to the dorsal insula, bilaterally activated in the same subjects (Cerf *et al.*, 1998; Faurion *et al.*, 1999). This result also suggested an integrative function for the ventral insula, possibly combining taste and olfactory information into flavor perception.

The two additional foci found in the right insula with the group analysis were localized in the dorsal insula, in the medial and posterior portions. These foci were also identified in fMRI studies in response to taste and to stimuli combining taste and lingual somatosensory components (Faurion *et al.*, 1998; Cerf-Ducastel *et al.*, 2001). Early activation (around 150 ms) of the dorsal insula near the circular sulcus in both hemispheres in response to taste was described with MEG, indicating primary taste projections in this superior and posterior part of the insula (Kobayakawa *et al.*, 1996; Murayama *et al.*, 1996). This area was predominantly activated in the right hemisphere by taste, as determined with PET (Small *et al.*, 1999), by electric taste, with fMRI (Barry *et al.*, 2000), and for combined presentation of taste and lingual somatosensory stimulation, with fMRI (Cerf-Ducastel *et al.*, 2001), thus suggesting a relationship between the predominance of activation in the right hemisphere and the somatosensory aspect of taste perception. In the present study odorants were presented in aqueous solution to the mouth of the subject. Olfactory perception was thus associated with lingual somatosensory perception of the liquid flowing onto the tongue. The presentation of olfactory stimuli in aqueous solution in the mouth is known to induce sensory confusion, resulting in the interpretation of the sensation as a taste rather than an odor (Murphy *et al.*, 1977). Activation of the superior part of the right insula, usually associated with gustatory perception, could reflect this confusion between taste and olfaction arising from somatosensory stimulation of the tongue.

Activation in the rolandic operculum

The rolandic operculum (the base of pre- and post-central gyri) is not considered a part of the olfactory system and was not consistently found activated in previous neuroimaging studies of direct olfactory stimulation with air flow. However, activation of this region in the present study was consistent enough to be detected in both the group and individual analyses in both the left and right hemispheres. Although the present experiment was not designed to study this activation in particular and thus does not allow for any definitive interpretation, it raises the question of the existence of a specific activation in the rolandic operculum related to the present mode of stimulation of the olfactory system.

The rolandic operculum comprises the portion of the

somatosensory homunculus representing oral structures (Van Buren, 1983; McCarthy *et al.*, 1993) and recent neuroimaging studies have shown activation of the rolandic operculum in response to mechanical stimulation of the mouth and tongue (Hari *et al.*, 1993; Sakai *et al.*, 1995; Pardo *et al.*, 1997; Nakamura *et al.*, 1998). Taste stimuli and lingual somato-gustatory stimuli have been shown to activate this region (Cerf *et al.*, 1998; Faurion *et al.*, 1998, 1999; Cerf-Ducastel *et al.*, 2001). Zald and Pardo demonstrated activation of the rolandic operculum in relation to swallowing and to lingual stimulation with water (Zald and Pardo, 2000a). These results suggest that the rolandic operculum may be activated by a series of sensory tasks involving the mouth and tongue. Activation of this region in the present study could be related to association between stimulation of the tongue by liquids and olfactory perception and could thus reflect the specific oral origin of the retronasal olfactory perception of odorized solutions.

Conclusions

1. Retronasal olfactory stimulation using aqueous solutions of odorants (Murphy *et al.*, 1977) resulted in activation of all cerebral areas activated by direct olfactory stimulation with odorized air or odorized cotton wands presented to the nostrils. This mode of presentation thus allows the study of olfactory cerebral processes.
2. This mode of presentation especially allowed detection of piriform cortex, hippocampus and amygdala, rarely detected by neuroimaging studies utilizing direct olfactory stimulation with odorized air. The specific dissociation between perception and breathing and/or the use of perception profiles for extraction of activations may have facilitated detection of the previously cited areas. Thus, the presentation of odorants through liquids in the mouth and the recording of perception profiles may be useful methods for studying central olfactory processes.
3. Presentation of odorants in aqueous solution to the mouth poses fewer logistic challenges in the fMRI environment than presentation with an olfactometer and allows more precise control over stimulation than presentation of odor on cotton wands.
4. Presentation of odorants in liquids to the mouth is a more realistic model for studying flavor than simultaneous presentation of taste to the mouth and odor to the nostrils. Activation of the dorsal insula and of the rolandic operculum, previously found related to taste perception and to lingual somatic stimulation, might be specific to the presentation of odorants in aqueous solution on the tongue.
5. This mode of presentation furthermore allows the presentation of odor alone, taste alone or taste-odor mixtures with exactly the same stimulation paradigm, thus permitting direct comparisons between modalities.

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