Cerebral Activation to Intranasal Chemosensory Trigeminal Stimulation

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Abstract

Although numerous functional magnetic resonance imaging (FMRI) studies have been performed on the processing of olfactory information, the intranasal trigeminal system so far has not received much attention. In the present study, we sought to delineate the neural correlates of trigeminal stimulation using carbon dioxide (CO_2) presented to the left or right nostril. Fifteen right-handed men underwent FMRI using single runs of 3 conditions (CO_2 in the right and the left nostrils and an olfactory stimulant—phenyl ethyl alcohol—in the right nostril). As expected, olfactory activations were located in the orbitofrontal cortex (OFC), amygdala, and rostral insula. For trigeminal stimulation, activations were found in "trigeminal" and "olfactory" regions including the pre- and postcentral gyrus, the cerebellum, the ventrolateral thalamus, the insula, the contralateral piriform cortex, and the OFC. Left compared with right side stimulations resulted in stronger cerebellar and brain stem activations; right versus left stimulation resulted in stronger activations of the superior temporal sulcus and OFC. These results suggest a trigeminal processing system that taps into similar cortical regions and yet is separate from that of the olfactory system. The overlapping pattern of cortical activation for trigeminal and olfactory stimuli is assumed to be due to the intimate connections in the processing of information from the 2 major intranasal chemosensory systems.

Key words: anosmia, nose, olfaction, pain, stinging

Introduction

In recent years, brain-imaging studies using positron emission tomography (PET) or functional magnetic resonance imaging (FMRI) have provided new insights into the processing of sensory information. Although much work has been done on olfactory mediated sensations (for review, see Gottfried 2006), it is interesting to note that cerebral activation following intranasal trigeminal stimulation has not been systematically addressed. In fact, there is only one FMRI study (Hummel et al. 2005) where CO_2 was used as a relatively selective stimulant of the trigeminal nerve (Fröhlich 1851; Stevens et al. 1982; Thürauf et al. 1991). In that study in 19 subjects, trigeminal stimulation activated the ventral insular cortex, midbrain, superior temporal gyrus, anterior caudate nucleus, and the lateral orbitofrontal cortex (OFC). These results suggested that processing of intranasal activation follows a neural pattern that is, at least to some degree, similar for both trigeminal and olfactory stimulation. This was explained on the basis of the intimate connections between the trigeminal and olfactory systems (Doty et al. 1978; Cain and Murphy 1980; Hummel and Livermore 2002).

The present study aimed to extend the previous work insofar as trigeminal stimuli were presented to the left and right nostrils separately. It was hypothesized that trigeminal stimulation would produce activation in the same areas as it had been observed previously. In addition, due to the strong relations between the chemosensory systems, we also expected activation in "olfactory" areas such as the piriform cortex, the OFC, or the gyrus rectus (Kettenmann et al. 2001; Savic 2002; Gottfried 2006) as it already had been shown, at least in part, when relatively selective trigeminal stimulation was used (Hummel et al. 2005). Although trigeminal stimulation can be expected to produce a stronger contralateral activation in terms of the overall lateralization of trigeminal sensations, a stronger activation of the right hemisphere was predicted (Hummel et al. 1995; Hari et al. 1997) similar to what has been reported for the olfactory system (for review, see Doty et al. 1997).

Materials and methods

Subjects

In order to exclude possible causes of smell dysfunction, subjects underwent a detailed otorhinolaryngological examination including nasal endoscopy. All of them maintained that they were in good health. Subjects also completed a standardized handedness survey (Oldfield 1971) to insure that they were all right handed. To ascertain normosmia, all subjects underwent extensive testing with the ''Sniffin' Sticks'' test battery (Kobal et al. 2000). The study was conducted according to the Declaration of Helsinki on Biomedical Studies Involving Human Subjects; all subjects provided written informed consent. In order to exclude sex-related variability, only men ($n = 15$) were included. Their age ranged from 23 to 59 years (mean age 35.3 years).

Stimulation

Stimulants included carbon dioxide (CO_2) and phenyl ethyl alcohol (PEA). Using a computer-controlled olfactometer (olfactometer OM6b; Burghart instruments, Wedel, Germany) (Kobal 1981), CO_2 was presented to the left or right nostrils (concentration of 60% v/v CO_2). For technical reasons, PEA was presented to the right nostril only (concentration 20% v/v of PEA-saturated air). Chemical stimuli were embedded in a constant flow of odorless air (8 l/min). Stimulants were delivered through tubing terminating in a nose piece (inner diameter 4 mm) inserted into the subjects' nostrils. Airflow and humidity were precisely regulated by the olfactometer (Hummel and Kobal 2001). CO_2 was chosen for trigeminal stimulation (Fröhlich 1851; Stevens et al. 1982; Thürauf et al. 1991), and the rose-like odorant PEA was chosen for olfactory stimulation (Doty et al. 1978). At concentrations above 30% v/v (stimulus duration 200 ms, total flow 8 l/min), CO_2 produces sensations like ''burning,'' ''stinging,'' or ''biting.''

A 30-s ''on'' 30-s ''off'' block design was used as an imaging paradigm. Stimulants were delivered for 1 s every 4 s during the 30 s ''on period.'' During the 30 s ''off period'', subjects received odorless air (AIR). The order of presentation was randomized across subjects. In this context, it seems important to note that preliminary experiments (Hummel T, Becherer A, unpublished data) in 4 healthy subjects indicated that intranasal administration of approximately 2.27 l of pure CO_2 over a period of 4 min (equivalent to 28 stimuli of 1 s duration, with an average concentration of 60% v/v CO_2, at a total airflow of 8 l/min) produced only marginal changes in blood pCO_2 and pH (average values ± standard deviation; pCO_2: before 44.7 ± 5.9, after 49.3 ± 3.9; pH: before CO_2 stimulation 7.39 ± 0.03, after CO_2 stimulation 7.35 ± 0.02).

Stimuli were not presented in synchrony with breathing. Subjects performed the velopharyngeal closure technique in order to restrict breathing through the mouth (Kobal 1981). Prior to testing, subjects were trained in this technique using biofeedback. This training was performed during an introductory session where subjects used a thermistor placed under their nostril. The use of this method also minimizes the occurrence of sniffing behavior in response to the stimuli.

Imaging procedure

The study was performed using a 1.5-T MR scanner (Sonata; Siemens, Erlangen, Germany). For anatomical overlays, a T_1-weighted (turboflash sequence) axial scan with 224 slices, voxel size of $1.6 \times 1.1 \times 1.5$ mm, a repetition time (TR) of 2130 ms, echo time (TE) of 3.93 ms, and 2 averages (2130/3.93/2) was acquired. FMRI studies were performed in the axial plane (oriented parallel to the planum sphenoidale to minimize bone artifacts) using a multislice spin-echo echo-planar imaging sequence. Scan parameters included a 64×64 matrix, voxel size of $3 \times 3 \times 3.75$ mm, TR of 3000 ms, and a TE of 35 ms. A total of 120 images were acquired at each of 24 slice locations per paradigm over the course of a FMRI scan of approximately 6 min length. The 3 imaging conditions consisted of CO_2 delivered to the right nostril (CO_{2R}), CO_2 delivered to the left nostril (CO_{2L}), and PEA delivery to the right nostril (PEA_R). Each task paradigm had its own low-level baseline (air) and consisted of 6 alternating rest–stimulus cycles (60 s each) over the 6 min.

Behavioral data analysis

Following each task paradigm of 6 min length, subjects verbally rated the overall intensity of the stimuli using either of two 11-point category scale (10 = extremely strong, 5 = moderately strong, and zero = no sensation). Subjects were instructed and trained in a prior session to rate the overall intensity of the trigeminally mediated sensations when receiving CO_2 and the odor intensity when smelling PEA (Figure 1). A paired t-test was performed in order to compare the average ratings of CO_2 when presented in either nostril. No significant difference in intensity was found between nostrils ($P < 0.05$).

Data analysis

Neuroimaging data were pre- and postprocessed using SPM2 (Wellcome Department of Cognitive Neurology, London, UK, implemented in Matlab 6.5 R13; The MathWorks, Inc., Natick, MA). Functional data were registered, motion corrected, and resliced using SPM2 preprocessing procedures. The resulting images were coregistered to the corresponding T_1 volumes. Analyses were done on spatially normalized (stereotactically transformed into Montreal Neurological Institute [MNI] ICBM152 space; MNI template supplied by SPM2) and smoothed images (a 7-mm full width at half maximum [FWHM] Gaussian kernel for individual analyses and a 10-mm FWHM Gaussian kernel for the group analysis). In the random effects analysis,
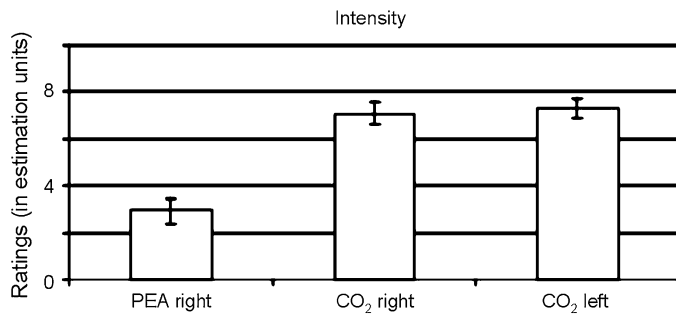
Figure 1 Mean ratings of overall intensity of CO_2 and PEA, respectively. Error bar signifies the standard error of the mean.

contrasted images were analyzed using a paired sample t-test to highlight the difference between conditions; effects were thresholded at $P < 0.001$ uncorrected with a cluster criterion of 3 voxels. For the conjunction analysis, results were also thresholded at $P < 0.001$, and an identical cluster criterion was used. No peaks survived a threshold of $P < 0.005$ corrected across the entire brain, and hence, all reported peaks are uncorrected.

Results

A direct contrast between stimulation of CO_{2R} with baseline (AIR_R; Table 1, $CO_{2R} - AIR_R$; Figure 2a) revealed significant activations in regions generally associated with pain (Borsook et al. 2004; De Leeuw et al. 2005): right precentral gyrus, left primary somatosensory cortex (SI), right secondary somatosensory cortex (SII), right insula, left cerebellum, and the left ventrolateral thalamus. The contrast also revealed "traditional olfactory regions" including left piriform cortex, left medial OFC, and right anterior OFC. In addition, the cortex around the left superior temporal gyrus, along the walls in sulcus, was activated.

The contrast between CO_{2L} and its corresponding baseline (AIR_L) revealed similar regions (see Table 1, $CO_{2L} - AIR_L$; Figure 2b). Predicted activations included the right brain stem, right dorsomedial and right ventral insula, right SII, left precentral gyrus, ventroanterior thalamus, centromedial thalamus, left cerebellum, and right superior temporal sulcus. The right piriform cortex and right anterior OFC were also activated.

The comparison of PEA_R with its corresponding baseline (AIR_R) is outlined in Table 1 ($PEA_R - AIR_R$) (Figure 3). Significantly activated regions included left rostral insula as well as primary and secondary olfactory regions such as the right amygdala and the right medial OFC.

Contrasts aimed at establishing unique activations for each nostril in response to stimulation with CO_2 were calculated (see Table 2, Figure 4). Results indicated that right-sided stimulation with CO_2 activated to a relatively higher degree the left superior frontal gyrus, bilateral cerebellum, and the left lateral OFC (Table 2); conversely, left-sided stimulation

activated the left superior temporal gyrus, right brain stem, and the left cerebellum (Table 2, $CO_{2L} - CO_{2R}$).

To determine if similar regions are activated in response to the 2 stimulus qualities, we carried out a conjunction analysis with the 2 contrasts of interest: $(PEA_R - AIR_R) \cap (CO_{2R} - AIR_R)$. The analysis highlights regions that are equally responsive in both conditions. That is, it identifies regions where there is a significant main effect of 2 contrasts while eliminating interactions between the simple effects. The conjunction analysis revealed activations in the facial area of the primary somatosensory cortices, right frontal operculum, and middle insula and bilateral activations in the medial frontal gyri (Table 3). The analysis also detected activation in the left piriform cortex, contralateral to the side of stimulation, as well as left medial OFC, a region thought to be the secondary olfactory cortex in man (see Table 3; Gottfried and Zald 2005).

Lastly, in order to stipulate neural regions preferentially involved in processing either a pure trigeminal or pure olfactory stimulus, we directly compared activations resulting from right-sided stimulation of CO_2 and PEA. A contrast between CO_{2R} and PEA_R indicated a superior involvement of the bilateral postcentral gyri and left middle cingulate, dorsomedial thalamus, brain stem, and anterior OFC in the processing of a pure trigeminal stimulus (Table 4, $CO_{2R} - PEA_R$). Conversely, the contrast between PEA_R and $CO2_R$ showed superior activation for traditional olfactory regions; namely, the right amygdala and medial OFC (Table 4, $PEA_R - CO_{2R}$).

Discussion

The present results indicate that trigeminal stimulation produces activation in areas typically involved in the processing of odorous information. These areas were 1) the contralateral piriform cortex considered to be the primary olfactory cortex (when disregarding the olfactory bulb as genuine primary olfactory cortex) (Zatorre et al. 1992), 2) the anterior OFC that is typically activated by odors (Tanabe et al. 1975; Rolls et al. 1996; Zald and Pardo 2000; Kareken et al. 2004), and 3) the rostral insula that has been found to be involved in odor quality discrimination (Savic et al. 2000). In addition, CO_2 activated the superior temporal gyrus, a region implicated in early cognitive processing of olfactory information (Kettenmann et al. 1996) as well as multimodal integration (Calvert and Thesen 2004). These findings are similar to areas reported previously following trigeminal activation (Hummel et al. 2005; see Introduction) with the major exception that, in addition to secondary olfactory regions, we also found activation in the contralateral primary olfactory cortices. In contrast, PET-based experiments using the mixed olfactory–trigeminal stimulus acetone (Savic et al. 2002) also reported the presence of additional activation in the amygdala, claustrum, anterior cingulate, and lateral hypothalamus. Whereas activation in some of these areas can

Table 1 Activations resulting from contrast between CO_{2R}, CO_{2L}, and PEA_R and their corresponding baseline (air)

Activated areas	K	Left-sided brain activation					Right-sided brain activation				
		x	y	z	Z	P^u	x	y	z	Z	P^u
Results from contrast CO_{2R} − AIR_R											
Precentral gyrus	95						48	−6	48	4.57	0.000
Superior temporal sulcus	104	−45	−42	12	4.44	0.000					
Rostral insula	12						33	−3	3	4.42	0.000
Postcentral gyrus (SI)	24	−60	−21	33	4.34	0.000					
Precentral gyrus	5						42	−15	25	4.25	0.000
Cerebellum	41	−51	0	39	4.16	0.000					
Ventrolateral thalamus	8	−33	−18	−48	4.06	0.000					
Piriform	11	−18	−12	3	3.84	0.000					
Anterior OFC	10	−24	48	−9	3.54	0.000					
Postcentral gyrus (SII)	5						21	58	−9	3.50	0.000
Medial OFC	8	−34	25	−16	3.25	0.001					
Results from contrast CO_{2L} − AIR_L											
Dorsomedial insula	121						36	−6	12	5.16	0.000
Brain stem (trigeminal nuclei region)	12						12	−39	−39	4.91	0.000
Postcentral gyrus (SII)	223						57	−6	24	4.42	0.000
Superior temporal sulcus	222						48	−6	24	4.42	0.000
Ventroanterior thalamic nucleus	47						12	−15	9	4.3	0.000
Centromedial thalamic nucleus	10						12	−18	−9	4.01	0.000
Precentral gyrus	18	−51	−9	45	3.9	0.000					
Ventral insula	5						39	12	−6	3.88	0.000
Cerebellar cortex	25	−18	−57	−27	3.74	0.000					
Anterior obitofrontal cortex	5						27	51	−9	3.56	0.000
Piriform cortex	5						24	9	−15	3.25	0.001
Results from contrast PEA_R − AIR_R											
Rostral insula	12	−42	12	0	4.36	0.000					
Amygdala	5						21	−12	−9	3.48	0.000
Medial OFC	5						20	48	−11	3.45	0.000

All reported activations were thresholded at $P < 0.001$ and were uncorrected (P^u). Coordinates of activated brain areas are presented in x, y, and z, separately for left- and right-sided brain activations. K is the cluster size. Z is determined from the voxel showing the maximal F value in each cluster.

be attributed to olfactory input (amygdala and hypothalamus; Gottfried 2006), activation in others is typically associated with nociceptive input (anterior cingulate; Hummel et al. 2005; Jantsch et al. 2005).

The presently observed patterns of activation can be explained partly by trigeminal relays to the amygdala via the lateral parabrachial complex (Bernard et al. 1989). Ascending fibers cross to the contralateral side; however, similar as in the olfactory system, some fibers also ascend ipsilaterally (Barnett et al. 1995). An additional way to ac-

tivate the olfactory system through trigeminal afferents may be due to the fact that "some trigeminal ganglion cells with sensory endings in the nasal epithelium also have branches reaching directly into both the olfactory bulb and the spinal trigeminal complex" (Schaefer et al. 2002). In addition, electrophysiological studies indicate that olfactory cell responses to chemical stimuli can be modified through the release of substance P and possibly other peptides (Lewis 1937; Holley et al. 1991; Raja et al. 1999) from trigeminal fibers innervating the olfactory epithelium (Finger et al. 1990; Kratskin
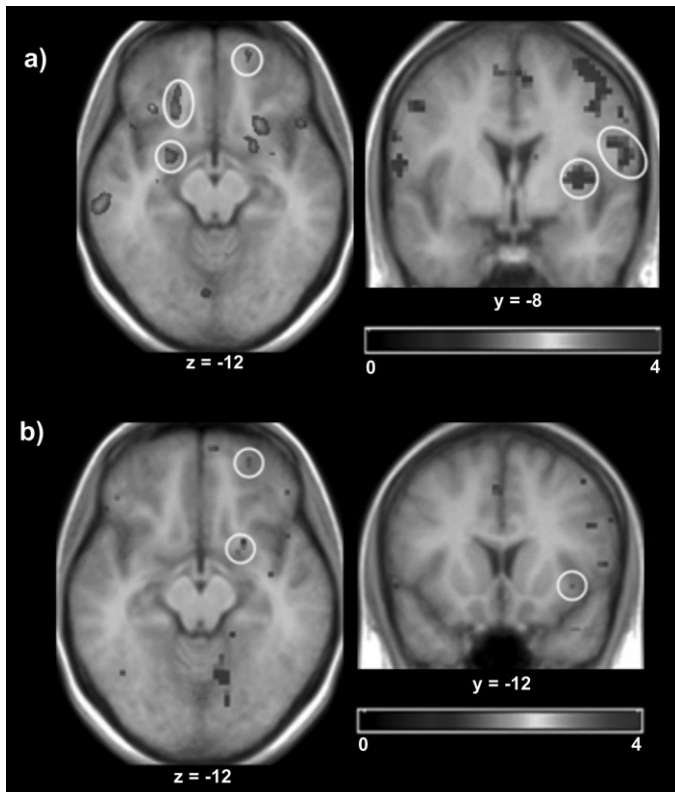
Figure 2   **(a)** Activations caused by contrasting CO_{2R} and its corresponding baseline (air): left piriform cortex, left medial OFC, right anterior OFC, right rostral insula, and right (SII). **(b)** "Traditional" olfactory areas activated in response to CO_{2L} stimulation versus its baseline: right piriform cortex, right anterior OFC, and right ventral insula. Location of coordinates is reported in neurological space where left-sided activations are displayed in the left hemisphere.
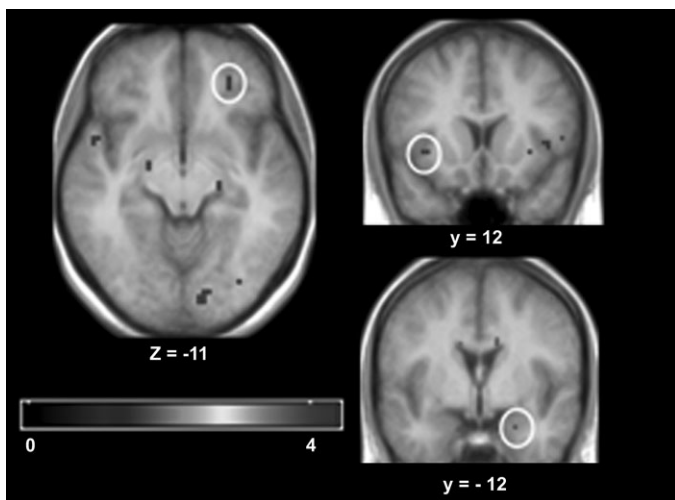

Figure 3   Regions activated by contrasting PEA_R with its corresponding baseline (AIR_R): right medial OFC, left rostral insula, and right amygdala. Location of coordinates is reported in neurological space where left-sided activations are displayed in the left hemisphere and similarly for right-sided activations.

et al. 2000) (see also Bouvet et al. 1987, 1988; Getchell et al. 1989).

Primary olfactory cortex

Although often reported as the primary olfactory cortex and believed to process basic sensory information, recent investigations have shown that the involvement of the piriform cortex in the processing of odors is more complex than previously assumed. In addition to its activation during passive smelling (Zatorre et al. 1992; Savic and Berglund 2004), it has been shown that the piriform cortex also contributes to emotional (Gottfried et al. 2002), cognitive (Dade et al. 2002; Plailly et al. 2005), and spatial information processing (Porter et al. 2005) related to odors and dissociation of odor quality and structure (Gottfried et al. 2006). In addition, the piriform cortex has been reported to be involved in sniffing without an olfactory percept (Sobel et al. 1998). The current piriform cortex activation appears not to be attributable to the processes mentioned above as CO_2 is virtually odorless and subjects performed the velopharyngeal closure technique, largely eliminating sniffing as a confounding variable. The activation could be related to the anatomical mechanism reported by Schaefer et al. (2002) where they showed that some branches of ganglion cells with sensory endings in the nasal epithelium project into the ipsilateral olfactory bulb in the rat. Based on the finding, one could postulate that this anatomical connection is responsible for the piriform cortex stimulation via the olfactory bulb and olfactory tract. If responsible, it would imply that activations of the piriform cortex were initiated in the periphery/olfactory bulb. However, if mediated by the periphery/olfactory bulb, the piriform activations should have been ipsilateral to the side of stimulation rather than contralateral, as it had been observed.

A more likely explanation is that the contralateral activation is a result of trigeminal input rather than a reflection of activation through the olfactory system (i.e., the olfactory bulb). This theory is supported by work in macaques using retrograde and anterograde axonal tracers (Ray and Price 1993), indicating that there are reciprocal projections between the dorsomedial nucleus of the thalamus and the piriform region.

Secondary olfactory cortex

Both medial and anterior OFCs were associated with right- and left-sided stimulation with CO_2 and were mostly contralateral to the stimulated nostril. The OFC has been shown in previous studies to be implicated in the perception of odors and is thought of as a secondary olfactory region (for review, see Zatorre and Jones-Gotman 2000; Gottfried and Zald 2005). Further, in comparison with the primary cortex, it is believed to be implicated in more complex aspects of olfactory analysis and integration. Anatomical evidence supports this hypothesis as neurons in the OFC receive inputs

Table 2 Activations resulting from contrast between nostrils during CO_2 stimulations

Activated areas	K	Left-sided brain activation					Right-sided brain activation				
		x	y	z	Z	P^u	x	y	z	Z	P^u
Results from contrast $CO_{2R} - CO_{2L}$											
Superior frontal gyrus	6	−18	9	66	4.84	0.000					
Lateral OFC	5	−25	21	−20	4.23	0.000					
Cerebellar cortex	11	−6	−49	−20	4.18	0.000					
Cerebellar cortex	6						13	−68	−22	4.11	0.000
Results from contrast $CO_{2L} - CO_{2R}$											
Superior temporal gyrus	8	−66	−45	6	4.16	0.000					
Brain stem	6						6	−24	−12	3.81	0.000
Cerebellar cortex	5	−18	−57	−30	3.32	0.000					

All reported activations were thresholded at $P < 0.001$ and were uncorrected (P^u). Coordinates of activated brain areas are presented in x, y, and z, separately for left- and right-sided brain activations. K is the cluster size. Z is determined from the voxel showing the maximal F value in each cluster.
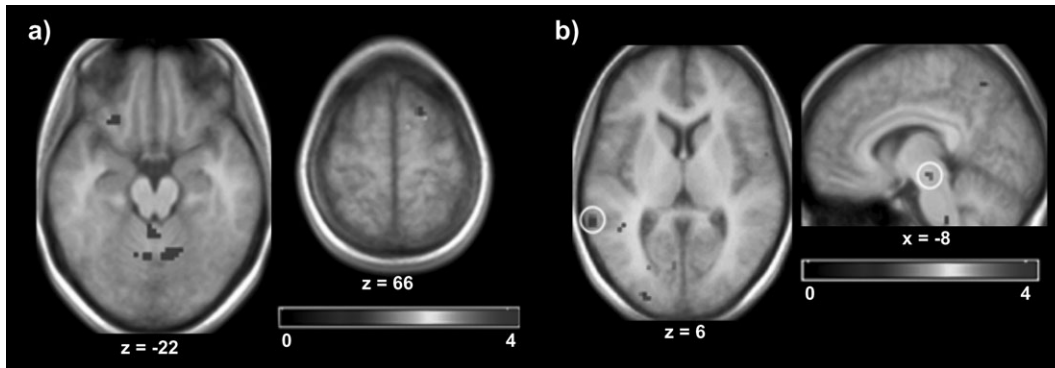

Figure 4   (a) Contrasting CO_{2R} with CO_{2L} indicate a relatively stronger left-hemispheric activation: left lateral OFC and bilateral cerebellar cortex. (b) The opposite contrast resulted in activation in regions such as the left superior temporal sulcus and the right brain stem in the region of the trigeminal nuclei. Location of coordinates is reported in neurological space where left-sided activations are displayed in the left hemisphere.

via the piriform cortex and the dorsomedial thalamus (Ray and Price 1993). The OFC has also been implicated in somatosensory processing including chemosensory trigeminal stimuli (Hummel et al. 2005). Taken together, it appears plausible that during chemosensory stimulation, the OFC may serve a specific function in the integration of both olfactory and trigeminal information.

Somatosensory regions

In addition to "olfactory regions," trigeminal stimulation of the left and the right nostril produced activity in the contralateral cerebellar hemisphere and thalamus. Right-sided stimulation yielded bilateral activation of the pre- and postcentral gyrus that was more pronounced in the left than the right hemisphere. Left-sided stimulation produced activation of the pre- and postcentral gyrus in the contralateral hemisphere, in addition to right-sided brain stem and activation of the ventrolateral, ventroanterior, and centromedial

thalamus. These regions have all been implicated in pain processing (Casey et al. 1996; Jantsch et al. 2005) and were partly reported in a previous study where CO_2 had been used (Hummel et al. 2005).

It should be noted that, although previous studies have shown the postcentral gyrus to be activated proportionally in response to nociceptive stimulation (Baron et al. 1999; Becerra et al. 1999; for review, see De Leeuw et al. 2005), neuroanatomical evidence suggests that SII is contributing to the encoding of painful stimuli (Bornhovd et al. 2002). It has been shown in primates that the region receives projections from the dorsomedial thalamus, which itself receives projections from the brain stem trigeminal nuclei (Craig 2004; Bowsher 2005). Furthermore, electrophysiological recordings and results from magnetoencephalographic studies (Chudler et al. 1985; Huttunen et al. 1986) demonstrated that SII is involved in the processing of nociceptive information.

Table 3 Activations resulting from a conjunction analysis with 2 contrast of interest: ($PEA_R - AIR_R$) ∩ ($CO_{2R} - AIR_R$)

Activated areas	K	Left-sided brain activation					Right-sided brain activation				
		x	y	z	Z	P^u	x	y	z	Z	P^u
Postcentral gyrus (SII)	47	−60	−3	12	4.61	0.000					0.000
Postcentral gyrus (SII)	24						60	3	18	4.41	
Frontal operculum	35						45	0	21	4.01	0.000
Middle insula	37						39	3	9	3.98	0.000
Medial frontal gyrus	6						57	6	42	3.94	0.000
Medial frontal gyrus	7	−48	42	6	3.93	0.000					
Medial OFC	6	−24	39	−9	3.86	0.000					
Piriform cortex	12	−24	4	−18	3.78	0.000					

Results from conjunction analysis with ($CO_{2R} - AIR_R$) and ($PEA_R - AIR_R$). All reported activations were thresholded at $P < 0.001$ and were uncorrected (P^u). Coordinates of activated brain areas are presented in x, y, and z, separately for left- and right-sided brain activations. K is the cluster size. Z is determined from the voxel showing the maximal F value in each cluster.

Table 4 Activations resulting from contrast between right-sided stimulation of CO_2 and PEA

Activated areas	K	Left-sided brain activation					Right-sided brain activation				
		x	y	z	Z	P^u	x	y	z	Z	P^u
Results from contrast $CO_{2R} - PEA_R$											
Postcentral gyrus	16	−51	−16	39	4.27	0.000					
Postcentral gyrus	14						48	−16	41	4.22	0.000
Middle cingulate	11	−5	−16	44	4.13	0.000					
Dorsomedial thalamus	9	−3	−18	0	3.52	0.000					
Brain stem	7	−2	−17	−16	3.98	0.000					
Anterior OFC	10	−24	48	−9	3.54	0.000					
Results from contrast $PEA_R - CO_{2R}$											
Medial OFC	13						15	16	−15	4.11	0.000
Amygdala	9						18	−5	−16	3.90	0.000

All reported activations were thresholded at $P < 0.001$ and were uncorrected (P^u). Coordinates of activated brain areas are presented in x, y, and z, separately for left- and right-sided brain activations. K is the cluster size. Z is determined from the voxel showing the maximal F value in each cluster.

As expected, right ventral and dorsomedial insular activation was observed in response to trigeminal and olfactory stimulation. It is known that several regions of this structure receive projections from the piriform cortex in rodents (Clugnet and Price 1987) and primates (Carmichael et al. 1994). In humans, insular activation in response to olfactory stimuli has been extensively reported, but specific insular regions of activation have varied between studies and conditions (Zald and Pardo 2000; Sobel et al. 2003; Djordjevic et al. 2005; Small et al. 2005). However, it has been reported that the anterior portion of the insula is implicated in attention toward painful stimuli (Brooks et al. 2002). In addition, 2 studies investigating the neural correlates of odorants with trigeminal–olfactory properties reported increased insular activity after contrasting a bimodal odorant with more selective olfactory stimuli (Yousem et al. 1997; Savic et al. 2002), and a third study investigating the neural correlates of a selective trigeminal stimulus also reported the ventral insula (Hummel et al. 2005). Hence, it seems reasonable to postulate that the insular activation observed in this study specifically relates to intranasal trigeminal chemosensory activation.

Nostril-dependent differences in the processing of trigeminally mediated sensations

Trigeminal stimulation in both nostrils appeared to specifically activate regions implicated in pain processing,

corresponding to the pre- and postcentral gyrus, and the ventrolateral, ventroanterior, and dorsomedial regions of the thalamus (Borsook et al. 2004; Jantsch et al. 2005).

Prior literature on the topic did address how CO_2 was processed birhinally (Hummel et al. 2005). Within the current data set, we were able to establish lateralized differences in terms of the processing of trigeminal information. However, these differences were not in line with the idea that right nostril stimulation produces stronger activation than left nostril stimulation (Hari et al. 1997). When CO_{2L} was contrasted with CO_{2R}, it resulted in greater activation in the posterior section of the left superior temporal gyrus, the left cerebellum, as well as the left brain stem, regions previously reported to be implicated in the neural processing of trigeminal/chemosensory stimuli (Hummel et al. 2005). Conversely, a direct contrast of CO_{2R} with CO_{2L} showed increased activation in the left superior frontal gyrus and the cerebellum. In addition, stimulation of the right nostril with CO_2 resulted in greater activity in the lateral OFC than left-sided stimulations. As well as being considered a secondary olfactory region, the lateral OFC has been implicated in olfactory-related stimulus integration. Gottfried et al. (2002) in a study on olfactory learning using classical conditioning of faces and appetitive, aversive and neutral odors reported the structure as being involved in olfactory–visual encoding independent of valence. Similarly, a study by de Araujo et al. (2003) showed that the odor of strawberry activated the lateral OFC when presented retronasally; a process related to odor–taste integration. The implication that the lateral OFC may play a role in odor integration with other senses appears at least anatomically plausible as it receives input from olfactory (Carmichael et al. 1994), taste (Rolls and Baylis 1994; Baylis et al. 1995), or visual systems (Rolls 2004), making it a potentially ideal site for olfactory-related sensory integration.

Neural correlates of odor perception

Right-sided olfactory stimulation revealed increased activation in areas typically known to respond to olfactory stimulation. We found activation in the right anterior OFC, right amygdala region, and bilateral rostral insula. Although piriform activation was not observed in the present study, this appears not to be unusual as many other studies on olfactory activation also reported a lack of response in this region (Levy et al. 1997; Yousem et al. 1997, 1999; Zald and Pardo 1997; Fulbright et al. 1998; Royet et al. 1999, 2001; Kobal and Kettenmann 2000; O'Doherty et al. 2000; Zatorre et al. 2000; Suzuki et al. 2001; Wiesmann et al. 2001). However, a large number of published papers have reported piriform activations (e.g., Zald and Pardo 1997). The lack of activation in the presence of activation of other olfactory structures remains puzzling at the least. Several hypotheses have been suggested including a fast habituation response of the piriform cortex (Poellinger et al. 2001) or the role of the piriform cortex in the recognition of odors but not in odor encoding (Dade et al. 1998).

Neural systems shared by olfactory and trigeminal chemosensory stimuli

A direct comparisons of the 2 chemosensory stimuli via a conjunction analysis revealed that both types of chemosensory stimuli share anatomical substrates within the primary and secondary olfactory cortices, in the facial region of both primary somatosensory cortices, and the insula. Additionally, activation in the right frontal operculum and bilateral medial frontal gyrus was observed (but as these regions were unpredicted, they were not considered significant).

The conjunction analysis further reinforced our prior findings in that both types of stimuli appear to activate synonymous regions of the piriform cortex. Although activation in the piriform cortex was only observed in the trigeminal contrast of the analysis, this conjunction analysis supports the notion that the piriform cortex was also implicated in the processing of PEA, though it was likely below our established level of significance at the level of the simple contrast. Our finding suggests that, consistent with other studies, the piriform cortex is important in olfactory perception (Zatorre et al. 1992) and, furthermore, in trigeminal perception.

Other regions of interest were also noted in the conjunction analysis including the medial OFC and the middle insula, a portion of the insula posterior and distinct from the ventral insula. As previously noted, the OFC receives projection from both the piriform cortex and the dorsomedial thalamus (Ray and Price 1993). As highlighted by the conjunction analysis, the OFC has a significant role in the central processing of olfactory and trigeminal stimuli. Lastly, results from the conjunction analysis support our previous finding in that the activation in the ventral insula observed by contrasting CO_{2R} with its corresponding baseline appears to be uniquely due to the trigeminal activation.

Neural systems unique to olfactory and trigeminal chemosensory stimuli

Finally, to highlight differences between how both types of stimuli are processed, we contrasted responses to right-sided stimulation with CO_2 and PEA. The results indicate that regions previously implicated in the perception of trigeminal stimuli (Hummel et al. 2005) were preferentially activated by CO_2 as opposed to PEA. These regions include the postcentral gyrus, thalamus, brain stem, and middle cingulate. The inverse contrast, to no astonishment, showed greater activation in the right amygdala and medial orbitofrontal gyrus, 2 regions highly associated with olfactory processing (Gottfried 2006). Also of interest are the hemispheric differences; CO_2 predominantly activating left-sided (contralateral) regions, whereas PEA gave right-sided (ipsilateral) activations. In spite of their restrictions, mentioned above, the latest pair of contrasts confirms that olfactory stimuli activate traditional olfactory regions more than trigeminal stimuli and vice versa. The exception to this rule may be the piriform cortex, which by omission appears to be equally

activated by pure olfactory and, surprisingly, a pure trigeminal stimulus, despite traditionally being considered primary olfactory cortex (Zatorre et al. 1992).

With regard to the comparison between olfactory and trigeminally mediated sensations, however, important caveats of the study need to be kept in mind. They relate to differences between CO_{2R} and PEA_R in terms of intensity, potential differences in pleasantness, and potential differences in the degree of desensitization. Further, a complete comparison between PEA and CO_2 would also have required that PEA would have been presented to both nostrils and not to the right nostril only.

Conclusions

Results from the present study support previous findings (Hummel et al. 2005) that stimulation of the olfactory and the intranasal trigeminal system produces overlapping cerebral activations. We also report contralateral primary olfactory cortex activation in response to CO_2. This leads to the elucidation of a trigeminal processing system that appears to recruit similar cortical regions and yet is separate from that of the olfactory system. Future comparative studies based on the present results will have to consider the different intensities typically produced by olfactory and trigeminal stimuli. In these studies, it will also be important to investigate potential differences in the degree of desensitization to trigeminal or olfactory stimuli, respectively. In addition, future studies may also focus on stimulus presentation in relation to sniffing (compare Bensafi et al. 2005).

Acknowledgements

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