Odor Processing in Multiple Chemical Sensitivity

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Abstract: Multiple chemical sensitivity (MCS) is characterized by somatic distress upon exposure to odors. As in other idiopathic environmental intolerances, the mechanisms behind the reported hypersensitivity are unknown. Using the advantage of the well-defined trigger (odor), we investigated whether subjects with MCS could have an increased odor-signal response in the odor-processing neuronal circuits. Positron emission tomography (PET) activation studies with several different odorants were carried out in 12 MCS females and 12 female controls. Activation was defined as a significant increase in regional cerebral blood flow (rCBF) during smelling of the respective odorant compared to smelling of odorless air. The study also included online measurements of respiratory frequency and amplitude and heart rate variations by recording of R wave intervals (RR) on the surface electrocardiogram. The MCS subjects activated odor-processing brain regions *less* than controls, despite the reported, and physiologically indicated (decreased RR interval) distress. In parallel, they showed an odorant-related increase in activation of the anterior cingulate cortex and cuneus-precuneus. Notably, the baseline rCBF was normal. Thus, the abnormal patterns were observed only in response to odor signals. Subjects with MCS process odors differently from controls, however, without signs of neuronal sensitization. One possible explanation for the observed pattern of activation in MCS is a top-down regulation of odor-response via cingulate cortex. *Hum Brain Mapp* 28:172–182, 2007. © 2006 Wiley-Liss, Inc.

Key words: environmental illness; hypersensitivity; odors; positron emission tomography; symptoms

INTRODUCTION

Functional somatic syndromes without identifiable pathophysiological changes are a major health issue [Wessely et al., 1999] and the need for validated quantitative measures and useful biological markers has been repeatedly emphasized [Kipen and Fiedler, 2002]. In particular, there is a call for research on how distress, which is triggered by environmental stimuli and mediated by specific peripheral nerves, is further processed by the brain. In this respect the syndrome Multiple Chemical Sensitivity (MCS) is of special interest, because it is related to a well-defined environmental stimulus, odor. Clarifying the odor-processing cerebral circuits in MCS therefore may add knowledge about possible mechanisms also behind other more complex and overlapping functional somatic syndromes attributed to environmental exposures [Levallois et al., 2002; Wessely et al., 1999].

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MCS is characterized by multisystem complaints attributed to exposure to low doses of odorous chemicals. The syndrome label MCS was originally proposed by Cullen [1987: p 657]. It referred to "an acquired disorder characterized by recurrent symptoms, referable to multiple organ systems, occurring in response to demonstrable exposure to many chemically unrelated compounds at doses far below those established in the general population to cause harmful effects." Individuals suffering from MCS will try to minimize exposure to common everyday odors in an attempt to avoid triggering or aggravating symptoms. In many cases, the condition leads to reduced work ability and quality of life due to extensive avoidance behavior. MCS is reported by an increasing number of persons, with a prevalence of self-reported MCS between 6% and 33% [Kreutzer et al., 1999; Meggs et al., 1996].

Although it is acknowledged that patients reporting MCS suffer from ill health and are clearly distressed, the mechanisms behind the symptoms are not known. The most frequently discussed etiologies are sensitization after high or repeated low exposure to chemicals [Bell et al., 1992, 1996], synesthesia where processing of olfactory stimuli would lead to trigeminal activation [Bascom et al., 1997; Meggs, 1995], a generally disturbed limbic processing leading to increased odor response [Bell et al., 1995; Dalton and Hummel, 2000], and neurogenic inflammation [Doty et al., 1988; Meggs and Cleveland, 1993]. In the case of neurogenic inflammation, chemicals are proposed to activate c-fibers of the trigeminal nerve, leading to a release of substance P.

Studies of MCS must take into account that signals from odorous compounds can be either purely olfactory (mediated by the olfactory nerve), olfactory + trigeminal, or pheromones. Brain imaging experiments have shown that olfactory stimuli are processed by the limbic system (amygdala + piriform cortex, orbitofrontal, inferior insular, and anterior cingulate cortex) [Savic et al., 2000; Sobel et al., 1998; Zald and Pardo, 1997; Zatorre et al., 1992]. Strongly trigeminal odorants, on the other hand, predominantly engage insular cortex, the dorsal portion of anterior cingulate, a minor portion of primary somatosensory cortex, brainstem, thalamus, and cerebellum [Savic et al., 2002]. Finally, two pheromone-like compounds, 4,16-androstadien-3-one, and estra-1,3,5(10),16-tetraen-3-ol, were found to cause sex, and sex orientation-differentiated activation of the sex hormone-releasing portion of the hypothalamus [Sobel et al., 1999; Savic et al., 2001, 2005]. Notably, both of these compounds are odorous when given undiluted and, thus, capable of eliciting an altered response in MCS subjects.

Considering this complexity of the neuronal circuits involved, an objective evaluation of general odor hypersensitivity or other type of abnormal odor processing will require studies of the entire brain with several odorants. In the present study we used positron emission tomography (PET) to measure changes in regional cerebral blood flow (rCBF) in 12 subjects with MCS and 12 controls during passive, birhinal smelling of various odorants. Of particular interest was to investigate whether MCS could be associated with neu-

ronal sensitization. Because neuronal sensitization implies that a given signal causes an enhanced neural response in the signal-processing areas, we hypothesized that MCS subjects would show an abnormal increase of cerebral blood flow in the olfactory brain. Another issue addressed was the possibility to detect olfacto-trigeminal synesthesia in MCS. Both olfactory and trigeminal odorants were therefore used. Finally, we tested whether a presumed abnormal response in MCS would be independent of the odorant type, and if such an abnormal reaction would be associated with altered processing not only in cerebral circuits triggered by the mere chemical compound, but also in the areas engaged by the immediate associations to this compound [Savic, 2002]. The set of odors used in the PET scanner, therefore, included, in addition to vanillin and acetone, also a male and a female putative pheromone [Savic et al., 2001] and four different odors that have been used in our previous studies [Savic, 2002; Savic and Berglund, 2004] and that are reported to frequently trigger distress in MCS persons. Thus, we selected both odorants that were very likely to evoke symptoms and those that were expected to be more neutral, such as vanillin, and the two pheromone-like compounds.

In addition to the pattern of cerebral activation, we also investigated the respiratory pattern and RR interval. An imbalance in the autonomic nervous system has been observed in other groups of idiopathic environmental intolerance, indicating sympathotone hyperresponsiveness to different stimuli [Lyskov et al., 2001; Sandstrom et al., 1997].

SUBJECTS AND METHODS

Subjects

Twelve healthy, heterosexual, nonsmoking MCS women (22–42 years, 32 ± 6) and 12 healthy, heterosexual, nonsmoking female controls (20–28 years, 26 ± 3) were investigated. All subjects had normal nasal anatomy (assessed by nasal inspection) and olfactory thresholds (assessed with the *n*-butyl alcohol test; see Jones-Gotman and Zatorre [1988]). The thresholds did not differ between the two groups ($P = 0.8$), MCS subjects $1.0 \pm 2.0 \times 10^{-4}$ M and controls $5.0 \pm 5.0 \times 10^{-5}$ M.

The persons reporting MCS were recruited by an advertisement in a local newspaper. The inclusion criteria used were based on the 1999 consensus report [Anonymous, 1999]. The participants were to have symptoms (which involved multiple organ systems) reproducibly triggered by exposure to low levels of multiple chemically unrelated and odorous chemicals. The condition had to be chronic (experienced for more than 1 year), but symptoms should improve or resolve with removal of the incitants.

All MCS subjects were working but actively avoided exposure to odorous chemicals. Four persons in the MCS group had a history of occupational exposure to chemicals. Four persons (including two with occupational exposure to chemicals) could identify an episode that triggered the first symptoms of MCS, but none reported any accidental toxic exposure. All MCS subjects reported that each one of the

following exposures triggered symptoms: diesel exhausts, gasoline, paint, nail polish remover, perfumes, and cigarette smoke. The most common symptoms were headache and nausea. The median duration of MCS was 6 years. None of the controls complained of odor sensitivity.

All the subjects had given their written consent and the study was approved by the Ethical Committee and the Radiation Safety Committee of the Karolinska Hospital and was performed according to the guidelines of the Declaration of Helsinki 1975.

Odors

The compounds used have been evaluated in several of our earlier studies [Savic et al., 2000, 2001, 2002]. They consisted of the typical olfactory odorant vanillin (VAN), (Sigma Chemical, St. Louis, MO), the odorant acetone (ACE) with strong trigeminal properties, and four other odorants, here denoted as OO. These four other odorants (cedar oil, lavender oil, eugenol, and butanol) were given during the same PET session (Sigma Chemical). With the exception of butanol (10% solution) and vanillin (97% solution), the odorants were undiluted. All were thus given at suprathreshold levels. The odor of ACE is usually perceived to resemble nail polish remover, the odor of cedar oil and lavender oil to be perfume-like, whereas butanol is perceived as a solvent.

We also used a male and a female putative pheromone [4,16-androstadien-3-one (AND), and estra-1,3,5(10),16-tetraen-3-ol (EST), respectively]. Although they are both odorous, heterosexual healthy women are shown to activate the olfactory circuits with EST, whereas with AND they primarily engaged the hypothalamus, and showed only subsignificant cluster in the olfactory circuits [Savic et al., 2001]. Because of this differentiated pattern it was of particular interest to investigate whether heterosexual women with MCS, being hypersensitive to odors, would show a significant activation of olfactory regions also when smelling AND.

Experimental Procedures

The PET system was Siemens ECAT Exact HR, with a spatial resolution of ~3.8 mm full-width at half-maximum (FWHM) run in a 3D mode. The PET tracer was ^{15}O -H $_2$ O. Because of the restrictions for radioactivity exposure, the maximum number of injections was 15, each containing about 12 mCi. OO odorants yield a strong signal-to-noise ratio [Bengtsson et al., 2001] and were presented once. ACE, VAN, AND, EST, and AIR were presented two to three times, balancing the number of repetitions between subjects and groups. The order of scans representing the respective condition was randomized. Due to sound disturbance outside the scanner room, or movement of the subject, eight scans were omitted in the final analysis.

One PET stimulation run lasted 80 s, but the real acquisition time was 60 s [Savic et al., 2000, 2001; Savic, 2002]. Each presentation started 5 s after the bolus injection and was indicated by touching the subject's right index finger. During OO, each of the four odorants was presented in a glass

bottle at approximately 10 mm distance from the nostrils, for 15 s, with an interval of 5 s of breathing the air in the scanner room between. The reason for using several odorants during the same scan was to increase the chance of exposing the subject to an odor that she would have an experience of being sensitive to. During AIR, ACE, VAN, AND, and EST the identical on/off mode was applied, but the stimulus was always the same item (odorless air or the respective odorant).

The general instruction to subjects was that they would smell an odor or odorless air. They were told to breathe passively (not sniff). This procedure was trained on several occasions prior to the PET experiments (but with other compounds). The subjects were also prompted to concentrate on the perception of the presented item, without trying to categorize or label it. They were uninformed about the identity/category of the item(s), or the order of conditions. After each scan they were, however, asked whether they smelled any odor or not [Savic et al., 2000, 2001, 2002].

Respiratory frequency and amplitude were recorded on-line during each scan using a strain gauge around the lower thorax (Comair AB, Stockholm). The amplitude was measured as the distance between the maximum inspiration and expiration and averaged over the session. Percent change in an index of respiratory volume (product of respiratory frequency and respiratory amplitude) was then calculated in relation to the resting condition for 1 min preceding the respective scan.

In order not to put too much distress on the subjects, only one on-line recording was performed during the PET measurements. The choice was to do the respiratory recordings online, but after the PET measurements we monitored heart rate by recording R-wave intervals on a surface electrocardiogram (RR intervals) continuously during three randomized exposures (duration 15 s) of AIR, VAN, ACE, AND, and EST with 60 s between each odor presentation. The mean RR interval during the 30 s immediately before exposure was used as baseline and the change in RR interval for each of the following heartbeats was calculated during the 30 s following each presentation. Finally, subjects scored the odorants for familiarity, pleasantness, intensity, and irritability using a 100-mm bipolar visual-analog scale [Savic et al., 2002].

Differences between MCS and controls (between factor) in the change in respiratory index with AIR, VAN, ACE, AND, and EST (within factor) were tested in a repeated measure analysis of variance (SuperANOVA, SAS Statistics, Cary, NC). In the case of a significant group*odor interaction, differences between groups for each odor were tested by calculating contrasts within the model. Population differences with respect to ratings of various odor characters and differences in change of RR during 30 s postpresentation intervals were tested accordingly.

Image Analysis

The PET images were filtered with a 10-mm Gaussian filter. They were corrected for possible head movements and

anatomically standardized by means of the Computerized Brain Atlas [Roland and Zilles, 1994] using the individual magnetic resonance images. The mean rCBF value was arbitrarily set to a flow value of 50 mL/min/100 mg.

Significant *activations* were calculated contrasting VAN, ACE, AND, EST, and OO with AIR, and *deactivations* by running these contrasts in the opposite direction. Both activations and deactivations were first calculated in each group using one group random effect analysis within the SPM 99 statistical package (Wellcome Dept. of Cognitive Neurology, London, UK). Group comparisons (controls and MCS subjects) were then carried out for each contrast, using two group random effect analysis (degrees of freedom (df) 21) with SPM99 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm99/>) [Friston et al., 1999]. Considering the results in our earlier studies that showed that activation with several of the odorants was rather limited [Savic et al., 2000, 2001, 2005], the T-threshold level was set to 0.01 and the *P*-level after the whole brain correction was set to <0.05 [Friston, 1991]. The only exception was the ACE vs. AIR contrast, in which the T-threshold level was 0.001 because of the very large and confluent clusters. Finally, we also compared the merged activation from all odors (ACE + VAN + OO + AND + EST vs. AIR) between the two groups.

In addition to the explorative analysis by SPM99, we also carried out specific hypotheses-based volume of interest (VOI) analyses. They had two purposes: (1) To investigate whether MCS subjects and controls had different normalized baseline rCBF in amygdala and piriform cortex. The amygdala VOI was delineated on both sides and included the lateral as well as the medial and central nuclei. It was first delineated on individual MR images [Watson et al., 1992], and the VOIs then transferred to the corresponding PET images. The VOI representing piriform cortex was outlined from orthogonal clusters obtained during VAN vs. AIR contrast in healthy females in one of our previous studies [Savic et al., 2001]. (2) To investigate whether MCS subjects when smelling vanillin activated cerebral regions that in our previous study were shown to be strictly involved in the processing of trigeminal stimuli [Savic et al., 2002]. For this purpose, three separate “functional” VOIs were applied. The first encompassed the posterior portion of anterior cingulate, the second primary somatosensory representation of face, and the third a brainstem cluster covering trigeminal nuclei. The outlines of these VOIs were determined from ACE vs. AIR contrast in a previously investigated group of healthy women [Savic et al., 2002].

For these calculations the rCBF was in all subjects first normalized to the global mean of 50 mL/min/100g. For comparisons of the baseline (AIR), the mean rCBF of all AIR scans was calculated in each VOI and subject. Differences between controls and MCS were tested in each predetermined VOI with two-way repeated measure ANOVA, using subject group as the between factor and the region as the within factor. In case of a significant interaction in a main effect the results were further explored with appropriate contrast. *P*-values were considered significant when <0.05.

Figure 1.

Odor ratings by MCS subjects and controls according to the VAS (vertical line). Error bars represent SEM. Statistically significant differences between groups are indicated. **P* < 0.05, ***P* < 0.01.

To evaluate whether vanillin-activated regions previously shown to process trigeminal odorants (see purpose 2), the mean rCBF for the three VAN scans and AIR scans was calculated in the respective VOI in each subject. Possible activations in each group were evaluated with paired *t*-tests (*P* < 0.05), and possible differences between the two groups with one-way ANOVA (*P* < 0.05).

RESULTS

Psychophysical Measurements

MCS subjects rated VAN and OO as more irritable, and ACE as more intense than controls. They also judged VAN and ACE as less pleasant than controls, and deemed OO less familiar than the controls (Fig. 1).

There was no statistically significant difference in the calculated respiratory index between the groups at baseline. Controls increased the calculated respiratory index during smelling of VAN and ACE and decreased this index during AIR, AND, EST, and OO. MCS subjects, on the other hand, increased this index during smelling of VAN, AND, and EST and decreased it when exposed to AIR, ACE, and OO (Fig. 2). The statistical analysis showed a significant group*respiratory index interaction (*F* = 3.8; *P* = 0.006; df 4). The two groups differed significantly with respect to acetone (*F* = 7.7; *P* < 0.01; df 1), and EST (*F* = 4.4 *P* < 0.05; df 1).

There was also a significant group*odor interaction with respect to change in RR intervals during different exposures

Figure 2.

Change in respiratory index (percent change in the product of frequency and amplitude during smelling minus the immediately preceding baseline product of frequency and amplitude) in MCS subjects and controls. Error bars represent SEM. Significant differences between groups are indicated. * $P < 0.05$, ** $P < 0.01$.

($F = 3.9$; $P < 0.004$, $df 4$) (Fig. 3). Whereas controls increased their RR interval (i.e., decreased heart rate) during presentation of all odors and AIR, the MCS subjects decreased their RR interval (i.e., increased heart rate) during smelling of VAN and ACE. They also showed a less pronounced increase in RR interval during smelling of AND and EST. Statistical comparison showed that MCS subjects exhibited an overall diminished RR response during exposure to odorants compared to controls ($F = 71$; $P < 0.0001$; $df 1$). The RR intervals were not recorded for OO.

Both controls and MCS subjects reported a strong perception of each of the presented odorants, which subjectively became weaker first after about 40 s of exposition.

Eleven MCS subjects, but none of the controls, complained of headache and nausea during odorant presentations. This reaction was observed in response to several different items, but was not consistent across subjects; with respect to AND, only one subject complained of distress.

PET Data

The pattern of activation was similar in MCS subjects and controls. Thus, smelling of VAN, EST, and OO activated amygdala, piriform cortex, sometimes including portions of insular and cingulate cortex (Tables I and II). AND activated the anterior hypothalamus in both groups, and right fusiform gyrus in MCS (Table II; Fig. 4C). Smelling of ACE engaged the posterior portion of anterior cingulate, the insular cortex, cerebellum, thalamus, and hypothalamus in both groups (Fig. 4B). The rCBF increased also in the lower brainstem (including the trigeminal nuclei), but reached a level of significance only in controls (Table I; Fig. 4B). Notably, when smelling VAN none of the subject groups showed clusters in the brainstem, or in the cerebellum, hypothalamus, posterior portion of the anterior cingulate, or the granular portion of insular cortex (Table I; Fig. 4A). Smelling of VAN thus did not engage regions processing trigeminal stimuli (also confirmed in the specific VOI analysis).

No deactivation was observed in MCS subjects during passive smelling of vanillin (AIR-VAN), passive smelling of

estratetraenol (AIR vs. EST), androstadienone (AIR vs. AND), or during smelling of OO (AIR-OO). Controls, on the other hand, deactivated occipital and parietal cortex with OO (Talairach coordinates 25 -61 28, -16 -80 13), and right frontal cortex with VAN (32 47 5). Both groups showed widespread deactivations of occipital, temporal, and parietal cortices with ACE (Table III).

The two-group random effect analysis showed that MCS subjects differed from controls in several aspects. First, they activated several odor-processing areas *less* than the controls (Tables I, II). This is evident both in the single group analyses and in the direct comparisons between the two groups. Smelling of AND, which primarily caused hypothalamic activations in both groups of women, was the only exception (Table I; Fig. 4). Second, during several conditions MCS subjects activated anterior cingulate and precuneus-cuneus, which was not observed in healthy controls (Tables I, II, IV; Fig. 4). These differences also appeared when all the odorous compounds were merged in a subject group comparison all odors - air (Table IV; Fig. 5). The activations of anterior cingulate in MCS subjects had somewhat diverse local maxima for the different conditions. To test whether these clusters were overlapping, a post-hoc conjunctive analysis was carried out for VAN, ACE, and AND. This analysis showed common clusters in anterior cingulate in subjects with MCS, but not controls ($P = 0.01$, corrected at $P < 0.05$, corresponding to coordinates -14 49 12 (and -8 40 4), $z = 4.3$, cluster size 2.4 cc, and in 22 20 -22 with $z = 5.2$, cluster size 1.3).

Because the respiratory pattern differed between MCS subjects and controls, the possible influence of respiration on cerebral activations was taken into account in a separate post-hoc analysis by using the respiratory index from each separate scan as a covariate of noninterest (SPM99, ANCOVA analysis) (Tables I, II). The general pattern of activation remained unchanged in both groups, as did the group

Figure 3.

Change in RR intervals (percent change in the RR interval during smelling minus the immediately preceding baseline RR interval) in MCS subjects and controls. Whereas controls increased their RR interval during presentation of all odors including AIR, i.e., decreased their pulse, the MCS subjects demonstrated a decrease during smelling of ACE and VAN and a less pronounced response during smelling of AND and EST. Error bars represent SEM. Significant differences between groups are indicated. * $P < 0.05$, *** $P < 0.001$.

TABLE I. Activations with common odorants

	MCS			Controls			MCS vs. controls			Controls vs. MCS		
	z-score	Size cm ³	Coordinates x, y, z	z-score	Size cm ³	Coordinates x, y, z	z-score	Size cm ³	Coordinates x, y, z	z-score	Size cm ³	Coordinates x, y, z
Vanillin (VAN)-AIR												
L amygdala + piriform cortex	3.5	2.8	-24, 4, -20	4.6	7.3	-18, -4, -22						
	3.5	1.1	-26, -28, -4	3.9	1.4	-24, 2, -20						
R amygdala + piriform + insular cortex	3.6	1.6	26, -8, -6	5.5	20.0	26, -2, -8				3.9	3.1	42, -4, -10 ^a
	3.9	3.2	24, -10, -6	3.6	3.2	22, -2, -2				3.8	2.0	38, -6, -6
L fusiform and lingual gyrus				4.4	4.5	-46, -46, -20						
				4.2	0.9	-16, -44, 4						
L insular cortex including BA44				5.6	11.2	-48, -2, 18						
				3.9	1.2	-50, 22, 16						
Anterior cingulate cortex	3.4	1.5	0, 20, -12				3.3	1.1	2, 48, -16			
	3.8	3.9	-4, 32, 0									
Aceton (ACE)-AIR												
L insular cortex (including a portion of precentral gyrus)	4.1	6.4	-18, 0, 0 ^a	5.2	5.0	-30, -6, 0						
	3.5	1.9	-38, 0, 6	4.9	1.6	-30, -8, 2						
R insular cortex (including portion of piriform cortex)	4.6	4.8	14, -12, -2	6.5	9.0	38, -10, 6				4.6	4.0	48, -4, -4
				4.2	1.6	26, -4, -6				4.1	1.6	48, -4, -4
L cerebellum				4.3	0.8	-48, -50, -28						
R cerebellum	6.2	1.8	8, -62, -24									
Hypothalamus + thalamus + brainstem	4.2	1.2	-4, -28, -2	3.5	2.8	16, -10, -20						
	4.0	0.5	-8, -30, -2	5.0	4.2	8, -32, -20						
				5.1	2.4	-2, -2, -6						
				4.4	0.5	4, -32, -26						
Anterior cingulate cortex ^a	5.0	4.8	-12, 44, 16	5.0	0.5	-8, 42, 12	4.3	1.6	-14, 34, 16			
	4.8	5.3	6, 42, 0				3.5	1.1	-32, 46, 8			
	3.9	1.2	-16, 48, 6						-16, 50, 2			
Other odorants (OO)-AIR												
R amygdala + piriform + cingulate + insular cortex	3.6	2.4	24, -2, -6	4.7	8.0	24, -2, -6						
	6.0	4.1	24, -8, -6	4.0	4.0	20, -8, -14						
L amygdala + piriform + cingulate + insular cortex	3.2	1.4	-14, -14, -8	4.2	13.0	-34, 0, 0						
	4.9^b	5.0	-26, 2, -14	3.4	8.8	-24, -4, -12				4.0	1.6	-20, -28, -6
Anterior cingulate cortex	3.3	1.8	4, 32, -4				3.2	4.0	20, 40, 32			
	6.2	4.3	-4, 30, 10				3.2	1.5	-18, 28, 24			
							4.5	2.0	-14, 48, 4			
Cuneus + precuneus	5.1	1.6	30, -88, -28				3.3	1.0	-8, -72, 30			

The MCS and Controls columns show clusters during odorant exposure vs. AIR. The other columns indicate regions that were significantly more activated in MCS subjects than in controls (MCS vs. Controls) and vice versa (Controls vs. MCS). The clusters were calculated with random effect analysis at $T = 0.01$ and a corrected $P < 0.05$ (except for ACE-AIR, where the T -threshold was 0.001). The anatomical localization of clusters is indicated by the regions they are covering and the Talairach coordinates for local maxima (x, y, z). Z-scores indicate the degree of activation. Although the local maxima in Control-MCS comparisons in several conditions indicate insular cortex, the clusters generally covered the amygdala and piriform cortex also. Boldface values refer to SPM analysis using respiratory index as a covariate, $T = 0.01$, corrected $P < 0.05$. L=left, R=right. BA = Brodmann area.

^a The local maximum was at the border of the cluster.

^b Confluent with activation of anterior cingulate

differences. The extent of activations, however, became generally smaller.

The mean normalized baseline rCBF (both sides included) did not differ between MCS and control subjects in amygdala (53.5 ± 2.5 in MCS vs. 54.6 ± 2.0 in controls), nor in the piriform cortex (64.7 ± 14.6 in MCS vs. 59.4 ± 3.1 in controls). Because our MCS subjects activated anterior cingulate more than controls, we wondered whether they could have a different baseline rCBF in this region. The normalized rCBF was therefore calculated in anterior cingulate in an

other post-hoc analysis. Anatomical VOI encompassing Brodmann areas 24, 32, and the subgenual portion of cingulate gyrus was delineated on each subject's MR images according to Takahashi et al. [2002; Savic et al., 2004]. In summary, this VOI was on coronal section bounded superiorly by the cingulate sulcus and inferiorly by the callosal sulcus. Its anterior border was the most rostral coronal plane containing the genu of corpus callosi, and the posterior was the most caudal coronal plane containing a clear view of the anterior commissure. After transferring the cingulate VOI to

TABLE II. Activations with estratetraenol (EST) and androstadienone (AND)

	MCS			Controls			MCS vs. controls			Controls vs. MCS		
	z-score	Size cm ³	Coordinates x, y, z	z-score	Size cm ³	Coordinates x, y, z	z-score	Size cm ³	Coordinates x, y, z	z-score	Size cm ³	Coordinates x, y, z
Estratetraenol (EST)-AIR												
R amygdala + piriform cortex				4.8	3.7	34, 0, -14				4.6	3.9	30, -8, -4
				3.8	3.0	34, 0, -12				3.7	1.9	34, -2, -6
Anterior cingulate cortex	4.9	4.8	-4, 20, -4	4.4	2.0	-8, 27, 42						
	4.0	1.2	-8, 46, 4									
Cuneus							4.1	3.6	8, -94, 10			
							3.7	1.8	8, -78, 6			
Androstadienone (AND)-AIR												
Anterior hypothalamus	4.0	2.0	2, -4, -2	4.3	2.3	-4, 0, -12						
			6, 4, -6	4.4	2.6	-6, 2, -10						
	3.4	1.6	8, 8, -8									
R piriform+insular cortex										3.8	2.4	36, 10, -12
Anterior cingulate cortex							3.8	0.9	-14, 48, 8			
							3.6	1.6	18, 54, -4			
R fusiform gyrus	3.9	5.3	40, -42, -12									
Cuneus							4.4	3.2	-16, -86, 18			

MCS and Controls columns show clusters during odorant exposure vs. AIR. The other columns indicate regions that were significantly more activated in MCS subjects than in controls (MCS vs. controls) and vice versa (Controls vs. MCS). The clusters were calculated with random effect analysis at $T=0.01$ and a corrected $P < 0.05$. Talairach coordinates for local maxima are shown as x, y, z . Z-scores indicate the degree of activation. L=left, R=right. Boldface values refer to SPM analysis using respiratory index as a covariate, $T=0.01$, corrected $P < 0.05$.

PET images acquired during the AIR condition, the mean rCBF in this region from the three AIR scans in each subject was compared between the two groups (unpaired t -test). No significant difference was found (the mean rCBF, mL/min/100g, was 67.2 ± 17.9 in MCS vs. 60.8 ± 3.2 in controls, $P = 0.1$).

DISCUSSION

The purpose of the present study was to investigate potential mechanisms behind hitherto unexplained symptoms attributed to environmental factors. We chose to study subjects reporting MCS because they suffer from symptoms similar to those in other idiopathic environmental intolerances, but have a well-defined and quantifiable reported triggering factor, odor. This circumstance allowed objective measurements of the possible underlying pathophysiology. The term MCS was used as a descriptive label of the present study group according to the definition of the Multiple Chemical Sensitivity report [Anonymous, 1999]. Since the prevalence of self-reported MCS is almost twice as high in females as in males [Kreutzer et al., 1999; Meggs et al., 1996], we chose to focus on females in this first study.

Contrary to the symptom description suggesting neuronal sensitization, subjects with MCS showed a *reduced* rather than enhanced activation of cerebral regions processing odor signals (Tables I, II, IV; Fig. 4). This difference was most pronounced in the insular cortex when using the two-group t -test, and in the amygdala + piriform cortex when using the separate one-group t -tests. Subjects with MCS differed from controls despite a normal ability to detect odorants, and the reported distress

during exposures to several odorants during the scans. The lower degree of activation cannot be attributed to impaired odorant-access, as no systematic reduction in respiratory index was present in MCS subjects (Fig. 2). Furthermore, we specifically tested this aspect by using the respiratory index from each separate scan as a covariate of noninterest. The pattern of activation in controls and subjects with MCS remained basically unaltered, as did the group differences (Tables I, II). Thus, differences in respiratory pattern cannot explain the observed differences in cerebral activation. The reason for low activation of the orbitofrontal cortex is that our subjects were trained to breathe passively and to not judge the odorants. Thereby, the cognitive component and the orbitofrontal activation were minimized.

How do the present results relate to the theory of neuronal sensitization in MCS? Theoretically, our findings may reflect a more efficient neuronal response to odors in MCS, resulting in an overall decrease in metabolic demand or in the total number of activated neurons. However, response efficiency is usually a result of training and previous exposure, but the groups did not differ with respect to their experience of the presented odors, they did not report a higher familiarity with these odors, and they had similar olfactory thresholds as the controls. Together with the observed pattern of activation, these data argue against neuronal sensitization as the mechanism behind MCS. Neither do they provide a physiological support for olfacto-trigeminal synesthesia, as the trigeminal system was not activated by the pure olfactory odorant vanillin, although it was perceived as clearly irritable (Fig. 4A). Also an *odor-unrelated* limbic dys-

Figure 4.

Activations with VAN (A), ACE (B), and AND (C) are shown in color. The color scale indicates z-values. To the right are images showing difference between controls and MCS subjects (up), and

MCS subjects vs. controls (down). Right hemisphere is to the right. The left cuneal cluster in the MCS-controls contrast for VAN-AIR was not significant.

function seems improbable as no significant differences between MCS subjects and controls were found in rCBF, respiratory index, or the RR interval when the stimulus was odorless air. Notably, because the presentation of stimuli was randomized the subjects were unable to anticipate whether they would smell an odorant or odorless air. Possible mechanisms of action must therefore link symptoms with the odorant stimulation. It is worth mentioning that almost all subjects reported their typical symptoms in response to several odorants presented during the scans. Furthermore, nail polish remover (resembling acetone), solvent odors (resembling butanol), and perfumes (resembling lavender oil) were by listed 10 of the subjects as the odorants that evoked headache and dizziness in their everyday lives. Thus, the items used in the study were relevant to the studied symptomatology. On the other hand, only one subject reported distress with AND, and yet the difference between the two groups was as with the other odorants. The distress in itself, therefore, cannot explain the consistently altered pattern of activation in MCS.

It has been reported in several studies [Anderson and Sobel, 2003; Zald et al., 1997] that odor intensity and valence

Figure 5.

Activations with all odors. Activations are shown in color and are superimposed on standard MR images. The contrast used is (VAN + ACE + EST + AND + OO) - AIR. The color scale indicates z-values. Right hemisphere is to the right.

TABLE III. Deactivations with acetone (ACE)

Regions with significant deactivations	MCS			Controls		
	z-score	Size cm ³	Coordinates <i>x, y, z</i>	z-score	Size cm ³	Coordinates <i>x, y, z</i>
Occipital + parietal cortex	5.6	42.2	-24, -68, 48	5.8	51.1	28, -60, 2
R temporal cortex	4.8	7.5	46, -4, -6	4.5	2.4	58, -30, -2
L temporal cortex	3.7	1.8	-52, -14, -8	4.2	1.5	-56, -26, 4
R parietal cortex				4.3	1.9	38, -38, 48

The clusters were calculated with random effect analysis at $T = 0.01$ and a corrected $P < 0.05$. Talairach coordinates for local maxima are *x, y, z*. Z-scores indicate the degree of activation.

L, left; R, right.

is mediated by different circuits, and that pleasant and unpleasant odors activate different portions of the orbitofrontal cortex. Considering that both odor pleasantness and intensity were perceived differently by the MCS subjects and controls, we conducted another post-hoc analysis in which we used the individual ratings of odor pleasantness and intensity for VAN and ACE (which showed the largest differences between the two groups) as covariates when evaluating the activations within and between the groups (SPM99, ANCOVA for VAN-AIR, and ACE-AIR). No changes were observed in the respective activations or group differences. Furthermore, we also investigated a possible dichotomy between lateral and medial orbitofrontal activation with respect to ACE-AIR and VAN-AIR using explicit orbitofrontal mask in the two-group *t*-tests. This mask covered the entire orbitofrontal cortex, on the right and left side separately, from $z = -20$ to $z = +10$ in the Talairach coordinate system. Thereby, we specifically investigated if MCS and controls differed in their activations of the orbitofrontal cortex during smelling of acetone and vanillin. No significant differences were observed despite lowering the level of significance to $T = 0.05$.

Notably, none of the groups showed significant activations of the orbitofrontal cortex. One reason could be that the subjects were specifically trained not to judge the stimuli, thereby minimizing the cognitive components during the process of perception.

The perception of an odorant is determined by integrating information of the volatile stimulus (bottom-up processing) with information that is stored in memory (top-down processing). Bottom-up processing relies almost exclusively on the data conveyed by the intensity or quality of the odor stimulus, whereas top-down processing makes use of information in memory, expectations, and even the perceiver's affective or emotional state. Two areas were almost consistently (although not always together) more activated in MCS subjects as compared to controls, namely, the anterior cingulate cortex (ACC), and precuneus-cuneus (Tables I, II, IV; Fig. 4). Major theories of ACC function have proposed a role in anticipation, attention, conditioning, harm avoidance, and perceptual selection [Devinsky et al., 1995]. ACC is also reported to mediate autonomic functions [Critchley et al., 2000]. Cuneus, on the other hand, is suggested to be highly involved in attention and the retrieval of episodic memories,

TABLE IV. Comparisons of activations in MCS subjects and controls for all odorous compounds included in the study.

All odorants-AIR	MCS vs. controls			Controls vs. MCS		
	z-score	Size cm ³	Coordinates <i>x, y, z</i>	z-score	Size cm ³	Coordinates <i>x, y, z</i>
L amygdala + piriform cortex				4.9	4.0	-18, -8, -20
				6.3	8.9	-24, 2, -20
R amygdala + piriform + insular cortex				4.7	8.1	20, 0, -8
				7.1	8.8	24, -2, -6
Anterior cingulate cortex	4.1	5.9	14, 30, 14			
	4.0	5.2	-22, 46, 8			
Cuneus + precuneus	4.0	6.1	28, -66, 42			
	3.9	4.8	26, -56, 10			
R parahippocampal gyrus	4.5	2.4	24, -46, -4			
L fusiform gyrus				5.4	1.0	-48, -58, -24

The columns indicate regions that were significantly more activated in MCS subjects than controls (MCS vs. controls) and vice versa (Controls vs. MCS). This analysis includes ACE, VAN, OO, AND, and EST, and uses AIR as the baseline condition. These clusters were calculated with random effect analysis at $T = 0.01$ and a corrected $P < 0.05$. Talairach coordinates for local maxima are *x, y, z*. Z-scores indicate the degree of activation. L, left; R, right. Boldface values refer to SPM analysis using respiratory index as a covariate, $T = 0.01$, corrected $P < 0.05$.

including odor memory [Andreasen et al., 1995; Fink et al., 1996; Maddock et al., 2001; Savic et al., 2000]. Attention and anticipation usually lead to an enhanced activation of areas mediating the anticipated function [Carlsson et al., 2000; Drevets et al., 1995]. In the present context, attention would be expected to result in enhanced, and not reduced activations of the odor-processing circuits, which was not found in our MCS subjects.

Another tentative mechanism is that the hyperactivation of the anterior cingulate and hypoactivation of the olfactory circuits in MCS reflected harm avoidance and stimulus selection. In subjects with MCS, harm avoidance could be conflict monitoring, related to a desire to diminish the perception of the odor, and opposed to the explicit instruction to continue smelling the odorous stimuli. In such a scenario the reduced activations of the olfactory regions may be an effect of a top-down modulation from the cingulate cortex. Although highly speculative, this alternative explanation to our results would be compatible with the observation of a significant group difference in the "emotional" portion of ACC [Bush et al., 2000; Vogt et al., 1995] (Tables I, II, IV)—an area suggested to mediate harm avoidance [Pujol et al., 2002]. Further studies, however, are required to specifically test this hypothesis.

CONCLUSIONS

Implications for Treatment

The present data indicate that MCS is linked to odor exposure. Contrary to the common view, no signs of neuronal supersensitivity were found. We propose that the underlying mechanism could be a top-down modulation of the odor-processing neuronal circuits.

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