3.3. Characteristics of Subjective Symptoms

Table 3 shows subjective symptom times and time ratios of respective symptoms for all subjects. These counted all symptom times in a 5-min interval including those during exercising, eating, and sleeping. Average time of symptom was 3.2 ± 3.0 (0.3–9.8) h. The order of frequency of symptoms is airway/mucous membranes (8/8 subjects), heart/chest-related (6/8 subjects), gastrointestinal (5/8 subjects), cognitive (4/8 subjects), neuromuscular (4/8 subjects), head-related (4/8 subjects), musculoskeletal (3/8 subjects), affective (3/8 subjects), skin (2/8 subjects), and genitourinary (1/8 subjects). Principal component analysis was performed on the variance-covariance matrix (8 subjects × 10 variables), where the variables comprised the time ratios of respective symptoms. Figure 2 shows principal component 1 (PC1) and principal component 2 (PC2) scores for each subject, which accounted for 78% variance. This result indicates the characteristics of symptoms of subject A, B, and H were different from other subjects.

Table 3. Subjective symptom time (h) and time ratio of respective symptoms (%).

Symptoms	Subject							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Symptom time	2.7	9.8	4.8	0.8	2.8	0.3	2.4	2.1
Musculoskeletal	0	8	0	0	52	0	0	4
Airway/mucous membranes	94	84	7	22	3	75	97	84
Heart/chest-related	0	84	24	44	0	25	17	92
Gastrointestinal	0	5	40	22	3	0	0	4
Cognitive	0	81	5	0	0	0	31	84
Affective	0	1	2	0	0	0	0	4
Neuromuscular	91	92	10	0	0	0	0	12
Head-related	94	92	43	0	18	0	0	0
Skin	0	2	0	0	0	0	0	64
Genitourinary	0	0	0	0	0	0	7	0

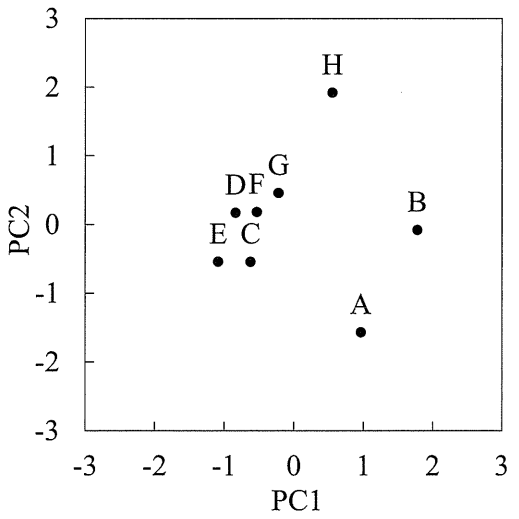

Figure 2. Plot of first principal component (PC1) and second principal component (PC2) scores of eight subjects.

3.4. Comparison between Subjective Symptom Time and Normal Time

Table 4 compares subjective symptom times and normal condition times. Because the symptom of subject F was observed for only one interval (5-min) after excluding confounding factors, the data is eliminated.

Table 4. Comparison of the parameter of subjective symptom time and normal condition time.

Parameters	Subject							↓ [f]	↑ [g]
	A	B	C	D	E	G	H		
VOC	↓ [a]	↑ **	↓	↑	↑ *	↑	↑	2 (0)	5 (2)
ΔVOC	↑ [b]	↑	↑	↓	↑ *	↑	↓	2 (0)	5 (1)
HF	↓ ** [c]	↓ **	↓ **	↓	↑ **	↑	↓	5 (3)	2 (1)
LF/HF	↑ * [d]	↓	↑ **	↑	↓	↓	↓	4 (0)	3 (2)
Temp	↓	↓	↑ *	↓	- [e]	↓ **	↓ **	5 (2)	1 (1)
RH	↑ **	↑ **	↑	↑	-	↑ *	↑ *	0 (0)	6 (4)

[a] ↓ Average value was lower during symptoms; [b] ↑ Average value was higher during symptoms; [c] ** Wilcoxon non-parametric test, $p < 0.01$; [d] * Wilcoxon non-parametric test, $p < 0.05$; [e] Data not obtained; [f] Number of the subjects showing lower during symptoms (significant); [g] Number of the subjects showing higher during symptoms (significant).

4. Discussion

4.1. Comparisons of VOC Exposure and HRV Parameters between Patients and Controls

It is assumed that patients avoid exposure to chemicals, and consequently their exposure level of chemical compounds is lower than that of healthy subjects [5]. However, the patients' VOC exposure concentration in total was higher than that of controls in this study although the differences were not significant (Table 1). The VOC concentration that were measured by the VOC monitor used in this study included various VOCs. Thus, chemicals that did not induce symptoms in patients may have been included in the measurements, which could explain why the VOC exposure concentrations of patients were not lower than those in healthy subjects. In addition, it should be taken into consideration that the subjects were recruited from volunteers and were not representative of a random sample of controls or MCS patients. With regard to HRV parameters, HF of patients had a low tendency although the differences were not significant.

4.2. Correlations between VOC Exposure and HRV Parameters

Significant negative correlation in two out of eight subjects and significant positive correlation in one out of eight subjects were observed between VOC concentration and HF (Table 2), suggesting there is no consistent trend between VOC concentration and HF for MCS patients. On the other hand, significant negative correlations between ΔVOC and HF were observed in four out of eight subjects, suggesting that changes of VOC concentrations in 5-min intervals were associated with decreased activity of parasympathetic nervous system. In addition, significant negative correlations between d + VOC and HF were observed in four out of eight subjects, and significant positive correlations between d − VOC and HF were seen in five out of eight subjects. These results indicate that HF tends to be low when the VOC concentrations increase or decrease. If VOC exposure is the cause of decreased HF, it is assumed

that decreased VOC exposure enhances HF power. However, HF was low when the VOC concentration decreased. Based on the existence of a causal relationship between exposure and the parasympathetic nervous system, one possibility is to assume that decrease of VOC exposure as with increase causes the HF decrease. Another possibility is that a delay of HF increases after VOC exposure. Between LF/HF and ΔVOC, significant positive correlation was observed in two out of eight subjects. Significant positive correlations were observed between d + VOC and LF/HF in one out of eight subjects, and significant negative correlations were observed between d − VOC and LF/HF in three out of eight subjects. These tendencies were opposite to HF.

As in the previous study, negative correlations between ΔVOC and HF in six out of seven healthy subjects were observed. Since these tendencies were more frequent in healthy subjects than in patients, there is a possibility that the absence of significant correlation between ΔVOC and HRV parameters is characteristic of MCS patients.

4.3. Comparison between Subjective Symptom Time and Normal Time

During subjective symptoms compared to normal conditions, VOC concentration was higher in five subjects and ΔVOC was higher in five subjects [significant differences were observed in subject B and E (Wilcoxon non-parametric test)] (Table 4). That is, VOC concentration and/or the change amount were high in all subjects except for subject F. In relation to HRV, the values of HF in five subjects were low during subjective symptoms (significant differences were observed in three subjects). These tendencies suggested the presence of high VOC concentration or change and low HF power when the subjects feel symptoms. In addition, the RH was high during subjective symptoms in six subjects, suggesting that RH has some relationship to subjective symptoms.

4.4. Case Studies

The measurement in this study was not designed to clarify the causal linkage between exposure and symptoms, but the context or simultaneity between exposure and symptoms can be observed from the time-series data. Therefore, time-series data for each subject were observed in detail in each case. To grasp the tendencies visually, average and maximum VOC concentration during 1 min and $\log_{10}$ HF during 1 min and 15-min moving average of $\log_{10}$ HF are indicated. Here it was assumed that the symptoms occurred when the subject sensed the exposure of chemicals. The longtime delay of symptom occurrence after exposure was not considered. This is based on the survey which clarified that the timing of symptom occurrence was almost immediately after an exposure [24].

Moreover, from this information, preventive measures were proposed for each subject. There is no common MCS treatment protocol accepted across medical disciplines. Gibson *et al.* surveyed perceived treatment efficacy for conventional and alternative therapies reported by a person with MCS. As a result, participants rated chemical avoidance, creating a chemical-free living space, and prayer as the three most useful interventions [25]. On the other hand, cognitive therapy, such as mindfulness, are being explored as treatment option for MCS [26,27].

This study includes several limitations attributed to various confounding factors based on the measurements in actual lives. VOC monitors measure the concentration of a wide variety of environmental VOCs in total, including non-symptom-related VOCs. Moreover, HRV parameters are

affected by various environmental factors and personal activities. In addition, the relationship between exposure and symptoms cannot refer to causal relationship. However, this method provided numerically-expressed data for actual condition of MCS exposure and symptoms which had been assumed depending on the interview and suggested a new insight into treatment processes.

4.4.1. Subject A

Subject A was a 39-year-old male and a researcher. Responsible exposures during monitoring were insecticides, tobacco, cosmetics, refresher, paint, detergent, smoke, and disinfectants inside a building. Figure 3 shows time-series data of VOC concentration and $\log_{10}$ HF of subject A. In the time from 18:30 to 0:30, several peak shape VOC concentration changes were observed and almost simultaneously (just before or immediately after the peak exposure) the subject felt the symptoms. This indicates that symptoms were induced when he was exposed to some VOCs (although symptoms before acute exposure may be anticipatory symptoms). It is noteworthy that $\log_{10}$ HF decreased before the occurrence of symptoms during exposure to relatively high and successive concentrations indoors (e.g., 21:00–21:30). From these findings, the exposure and symptom relationship is assumed as below: when HF is decreasing, a symptom is induced along with sudden elevation of VOC concentration. Meanwhile, a symptom occurred without high concentration of VOC, for example, during 16:20–17:10.

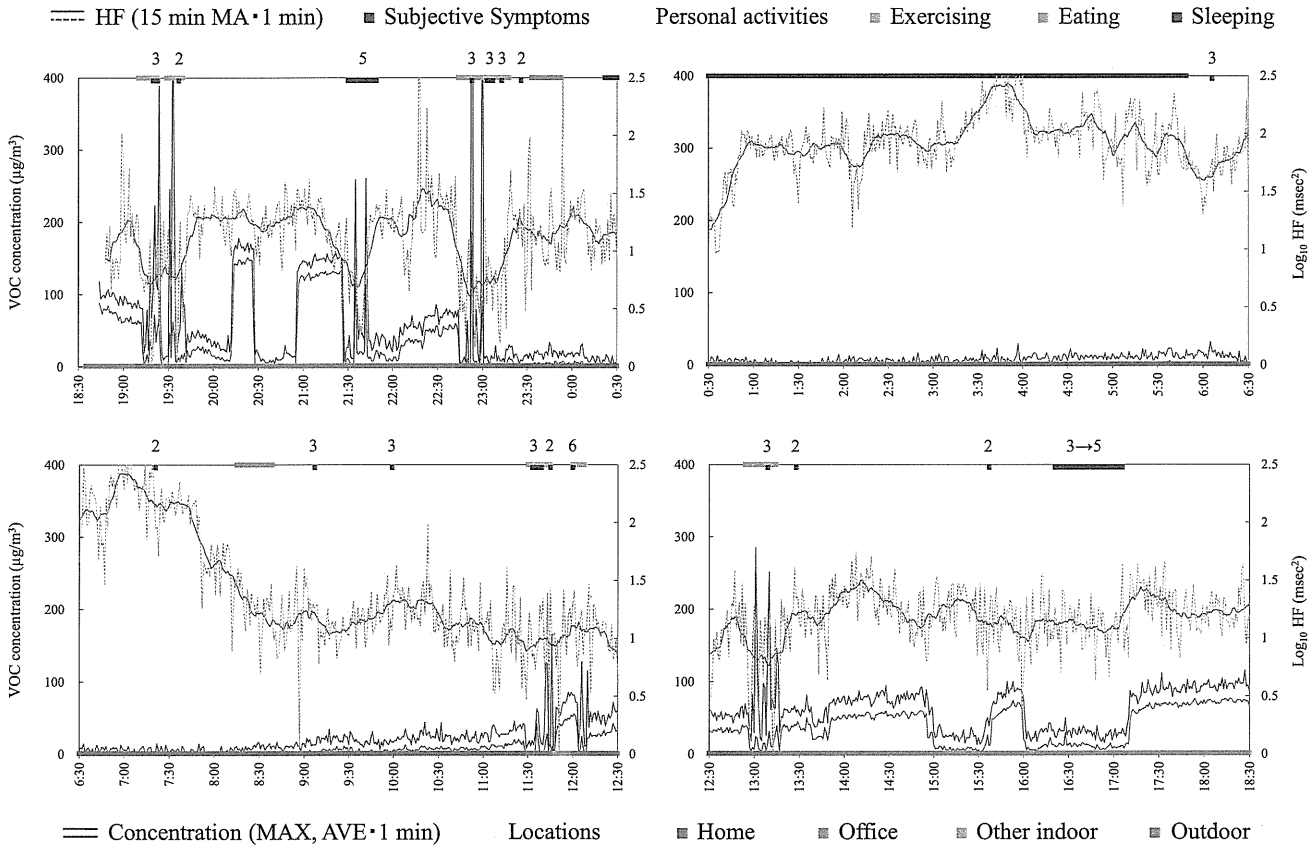

Figure 3. Time-series data of VOC concentration and $\log_{10}$ HF (subject A). Subjective symptoms and personal activities are indicated in upper side and locations are indicated in lower side. The values on subjective symptoms are symptom levels.

Consequently, since the symptom of this subject seemed synchronized with acute exposure, the measure to prevent symptoms for this subject is to avoid the condition or environment where the acute exposure of chemical or the environment (particularly outdoors) occurs. In addition, enhancing the parasympathetic nerve activities in daily life may be effective.

4.4.2. Subject B

Subject B was a 62-year-old female and a homemaker. Figure 4 shows time-series data of VOC concentration and $\log_{10}$ HF of subject B. During 8:00–8:30, a peak exposure was observed and at the same time symptoms were induced. However, the symptoms occurred frequently during 8:30–13:00, although the concentration of VOC hardly fluctuated. Therefore, in this case it was suggested that the subject mostly felt symptoms without increased VOC concentration. This suggests that the VOC monitor could not measure the concentration or concentration change of the chemicals that induced symptoms in this subject. For example the VOC monitor used in this study cannot detect formaldehyde which was rated as causing most symptomatology in persons self-identified with MCS [28]. Alternatively, this suggests that the subject repeated learned symptoms that could have been induced by a similar environment or condition in which symptoms were previously provoked by specific chemicals. HF during symptoms was significantly lower than that during normal conditions (Table 4). From this standpoint, other measures except for avoiding exposure, such as neurological treatment or cognitive therapy, may be effective.

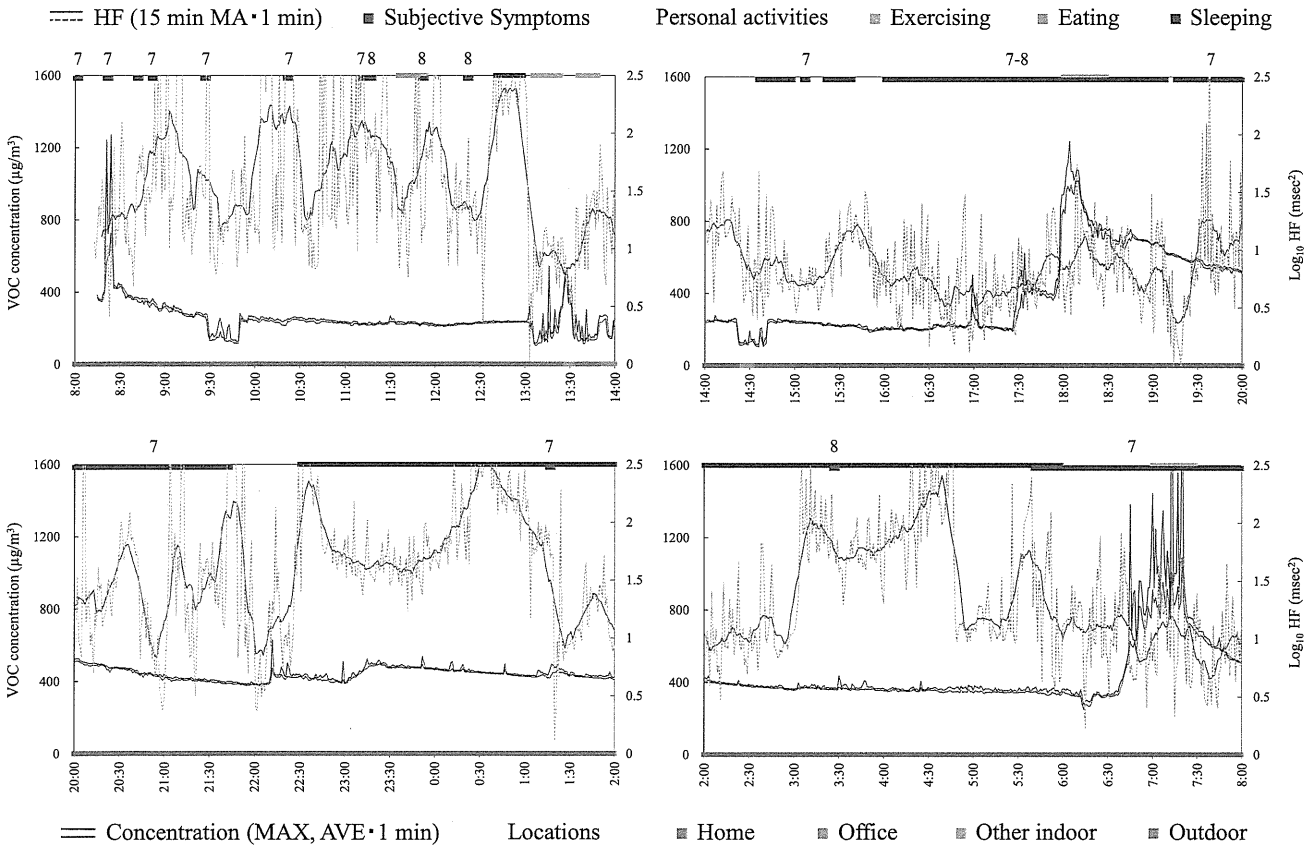

Figure 4. Time-series data of VOC concentration and $\log_{10}$ HF (subject B). Subjective symptoms and personal activities are indicated in upper side and locations are indicated in lower side. The values on subjective symptoms are symptom levels.

4.4.3. Subject C

Subject C was a 33-year-old female and a homemaker. Since symptoms worsened during measurement, the Holter monitor was removed at 19:30. Responsible exposures during monitoring were car exhaust, open burning, pesticide, tobacco, personal computer, and stove. Figure 5 shows time-series data of VOC concentration and $\log_{10}$ HF of subject C. The concentration in the house was relatively higher than that in other environments because the subject lived in new residential housing. In fact, measurement of VOCs in breath in the clean room indicated a high concentration of α-pinene, suggesting that α-pinene emitted from new wood material was absorbed by the body. Since the symptoms were not always induced in the house, it was suggested that VOCs existing on a steady basis in the house, including α-pinene, were not possible compounds for this subject. At 14:45 a symptom was induced in a car by open burning and almost simultaneously increase in VOC concentration was detected. Severe symptom was induced at 18:40 when a guest came. The concentration of VOC rapidly decreased and increased. This may be because of incursion of outdoor air by opening the front door. Because VOC concentration of outdoor air was low compared with indoor air, the concentration increase by responsible compounds could not be detected. This is a limitation of the measurement using the VOC monitor, which cannot separate the components.

HF during symptoms was significantly lower than during normal condition (Table 4) and some symptoms seem to be induced after HF decreases. Therefore, taking care to live a life enhancing the parasympathetic nerve activity may be effective also for this subject.

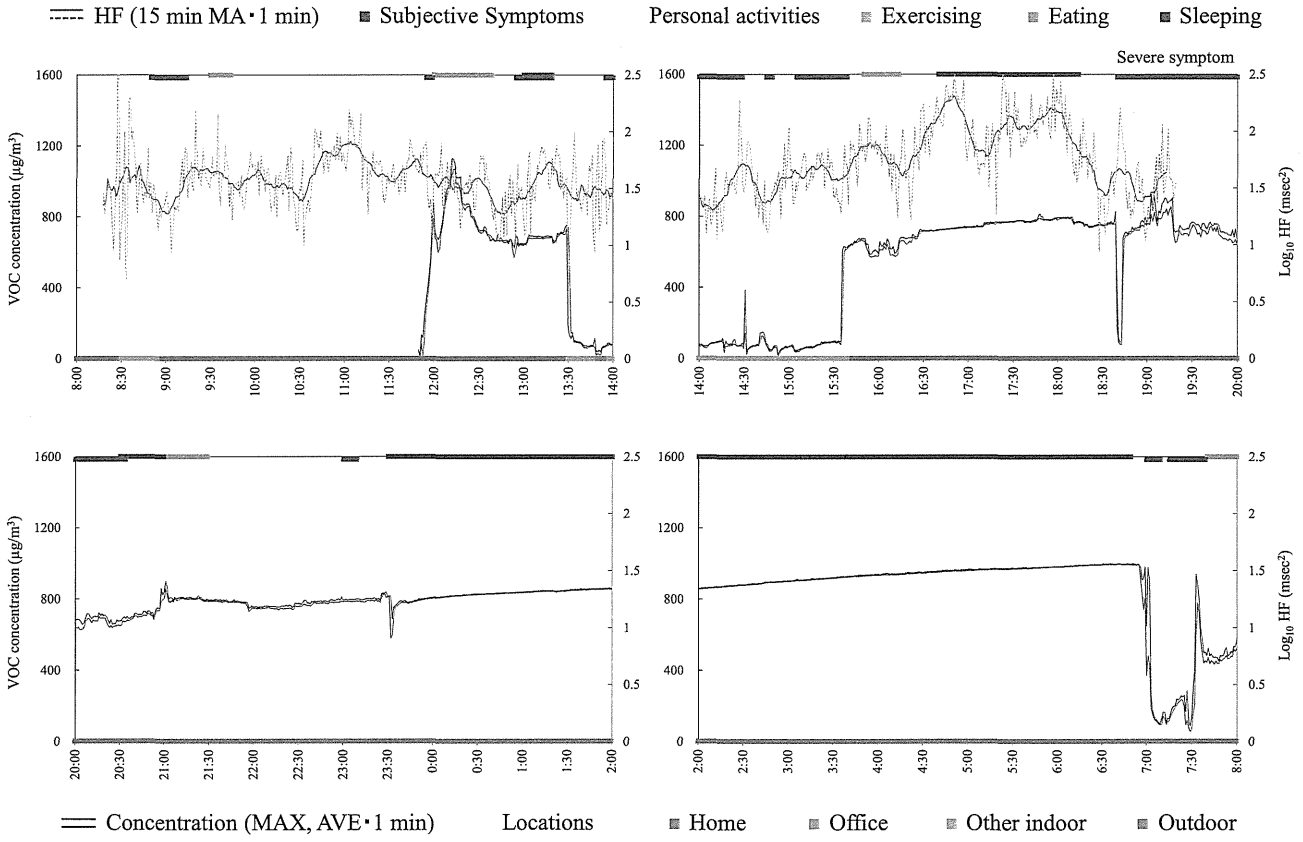

Figure 5. Time-series data of VOC concentration and $\log_{10}$ HF (subject C). Subjective symptoms and personal activities are indicated in upper side and locations are indicated in lower side. The values on subjective symptoms are symptom levels.

4.4.4. Subject D

Subject D was a 46-year-old female and a homemaker. Figure 6 shows time-series data of VOC concentration and $\log_{10}$ HF of subject D. After 20:00, the subject developed symptoms of flu. As is the case with subject A, sets of tendencies (after HF decrease, the subjective symptom, and acute increase of VOC concentration) were observed during 13:30–14:00 and 15:30–16:30. Therefore, avoiding the condition or environment of acute exposure and enhancing the parasympathetic nerve activities may be effective for prevention of symptoms.

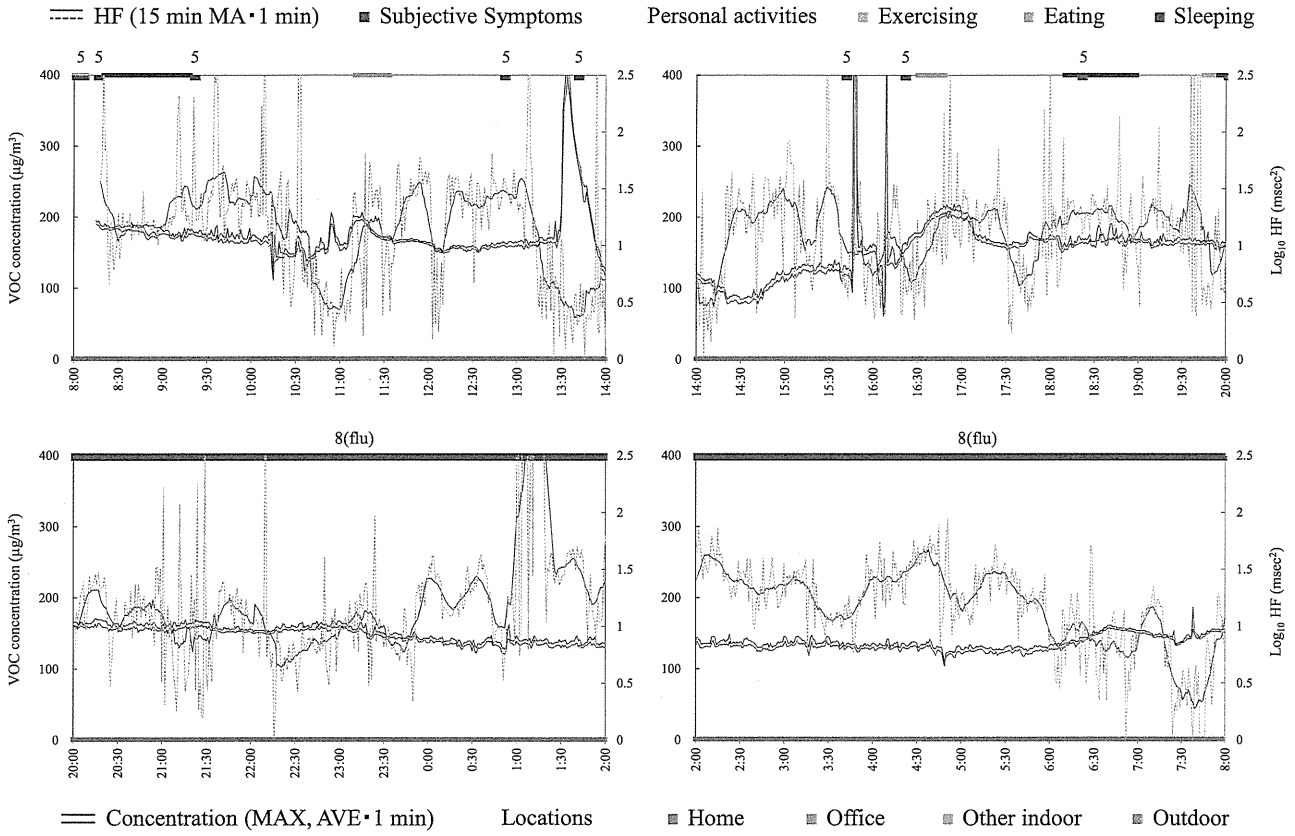

Figure 6. Time-series data of VOC concentration and $\log_{10}$ HF (subject D). Subjective symptoms and personal activities are indicated in upper side and locations are indicated in lower side. The values on subjective symptoms are symptom levels.

4.4.5. Subject E

Subject E was a 31-year-old male and an office worker. Figure 7 shows time-series data of VOC concentration and $\log_{10}$ HF of subject E. HF was significantly high during symptoms (Table 4). The symptom from 11:00 was induced while the VOC concentration was increased in office. Therefore, creating a chemical-free living space may be necessary.

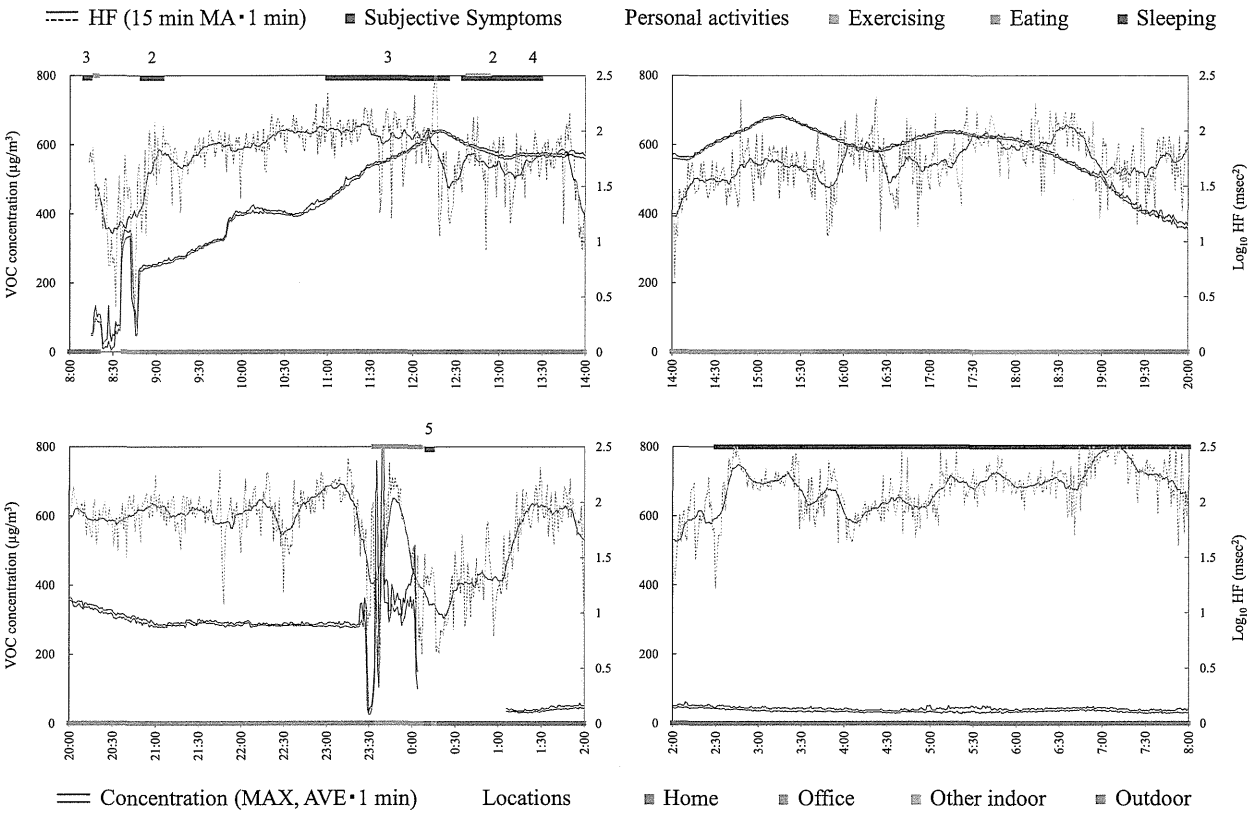

Figure 7. Time-series data of VOC concentration and $\log_{10}$ HF (subject E). Subjective symptoms and personal activities are indicated in upper side and locations are indicated in lower side. The values on subjective symptoms are symptom levels.

4.4.6. Subject F

Subject F was a 49-year-old male and an office worker. Responsible exposures during monitoring were liquid detergents used for cleaning and tobacco. Figure 8 shows time-series data of VOC concentrations and $\log_{10}$ HF of subject F. The symptoms were induced by relatively low concentrations at 9:40 and 13:40. After HF was decreased by exercise, the subjective symptom appeared during slight change of VOC concentration at 20:45. Meanwhile, symptoms were not induced by VOC concentrations by large increase and fluctuation from 15:30 and from 18:00. Therefore, avoiding the responsible exposures, such as detergents and tobacco, may be effective to prevent the symptoms for this subject.

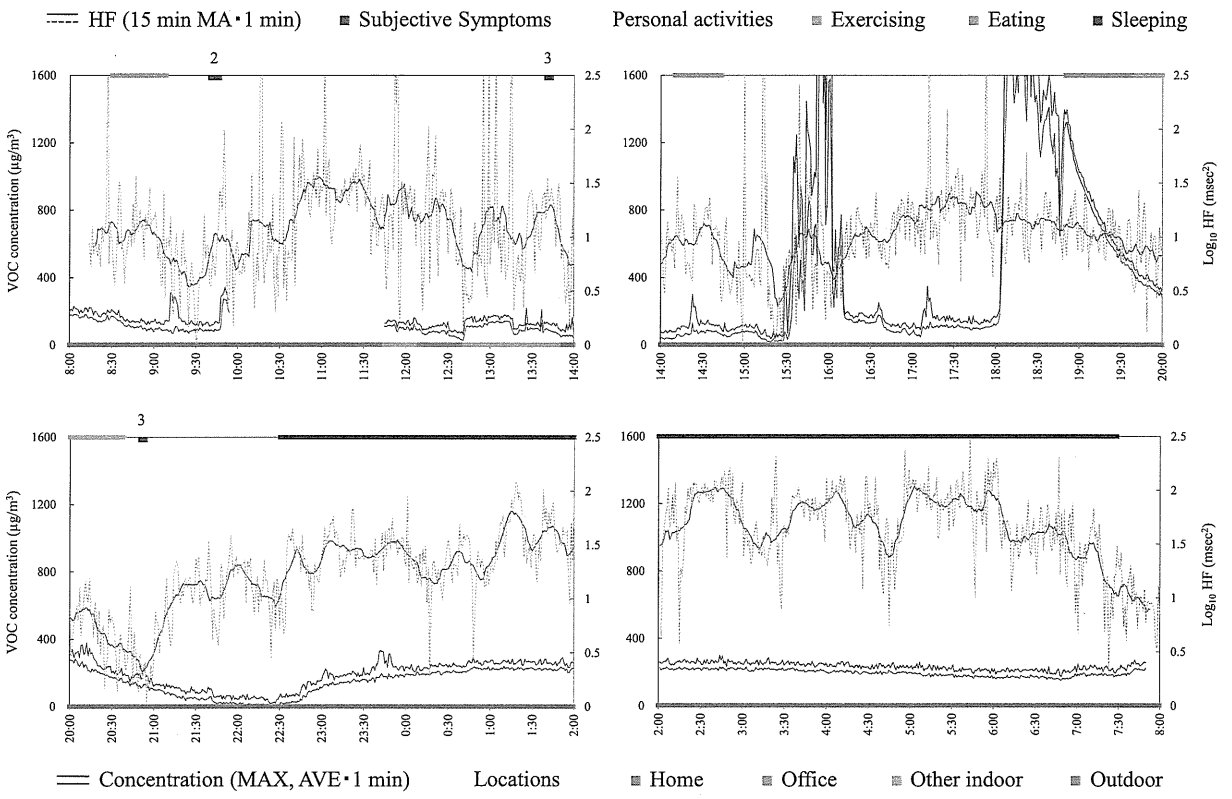

Figure 8. Time-series data of VOC concentration and $\log_{10}$ HF (subject F). Subjective symptoms and personal activities are indicated in upper side and locations are indicated in lower side. The values on subjective symptoms are symptom levels.

4.4.7. Subject G

Subject G was a 35-year-old female and on leave from her job. Responsible exposures during monitoring were odor of new organic cotton, bag for measurement apparatus, print, paper, pencil, ballpoint pen, envelope, odor of detergents, odor of drugstore, odors of cosmetics, perfume, and tobacco, odors of clothes and hair dressing, exhaust gas, smoke, fragrance, and odors of the dryer and closet. Figure 9 shows time-series data of VOC concentration and $\log_{10}$ HF of subject G. VOC concentration was very high during daytime, in the home, after breakfast (8:50). Consistent tendencies were not observed between VOC exposure, symptoms, and HF. Because the symptoms in this subject were mainly induced by odors of consumer products, which may affect only a local space and may not reach to the monitor sufficiently, there was a possibility that the monitor could not detect their effects.

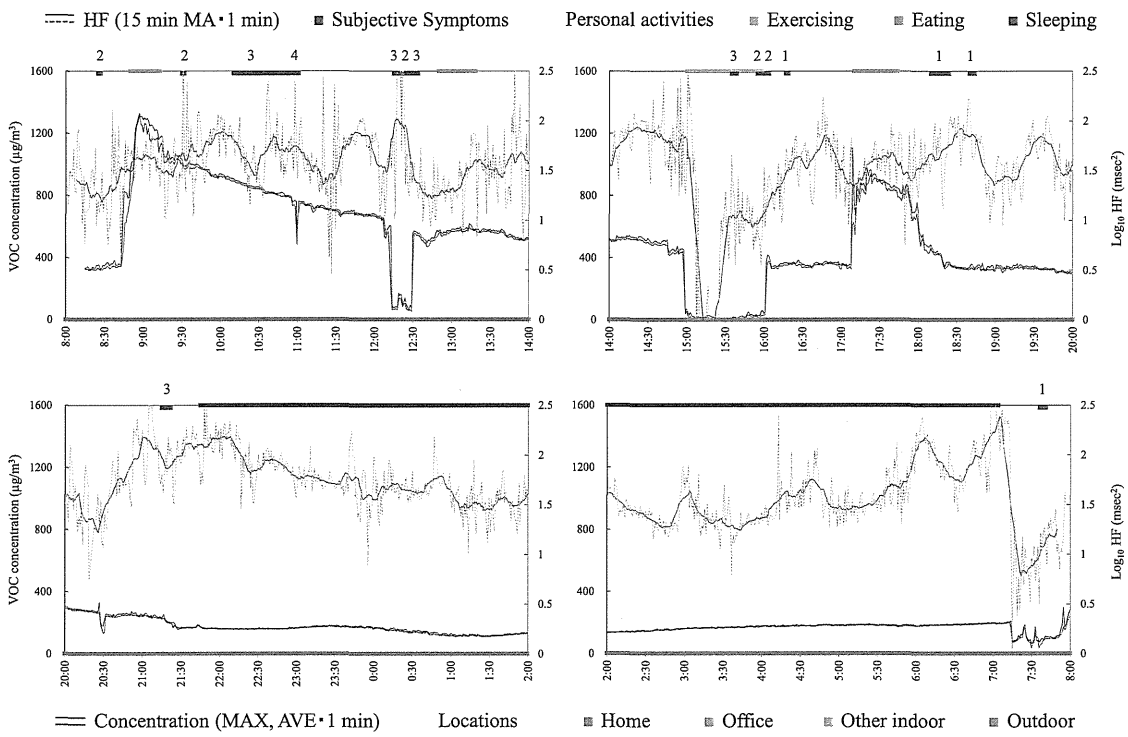

Figure 9. Time-series data of VOC concentration and $\log_{10}$ HF (subject G). Subjective symptoms and personal activities are indicated in upper side and locations are indicated in lower side. The values on subjective symptoms are symptom levels.

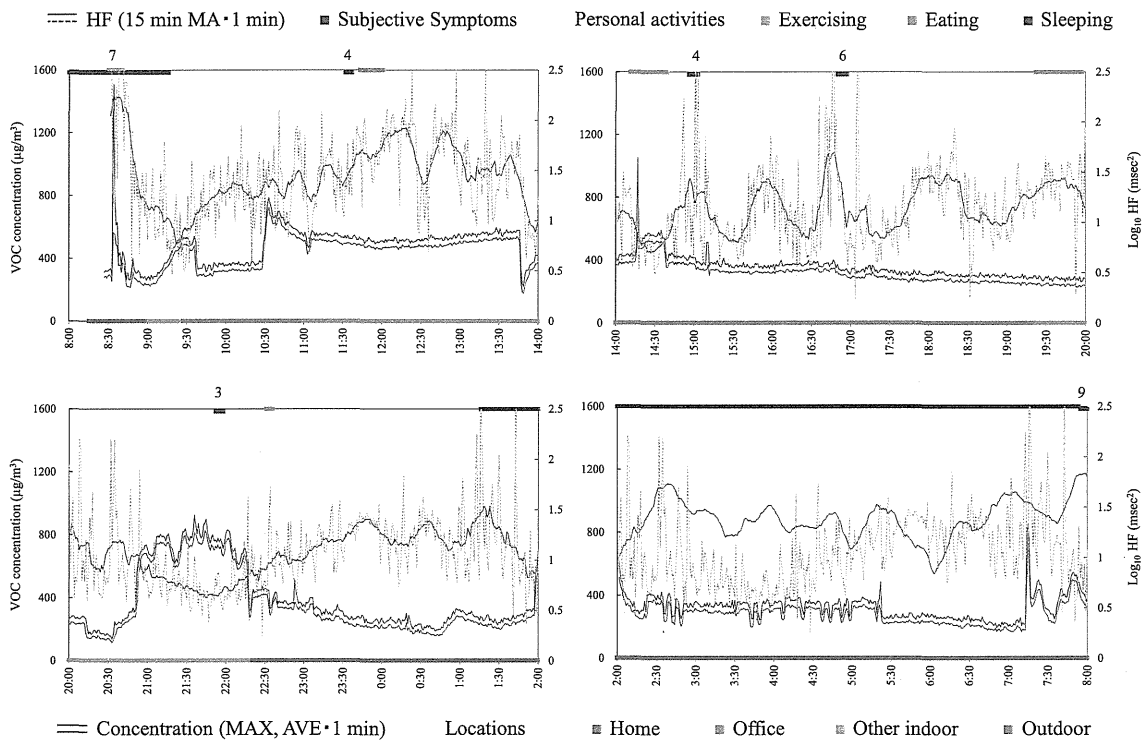

Figure 10. Time-series data of VOC concentration and $\log_{10}$ HF (subject H). Subjective symptoms and personal activities are indicated in upper side and locations are indicated in lower side. The values on subjective symptoms are symptom levels.

4.4.8. Subject H

Subject H was a 54-year-old female and a company owner. Figure 10 shows time-series data of VOC concentration and $\log_{10}$ HF of subject H. After decrease in HF, the subjective symptom appeared during relatively high concentration at 21:55. For other symptoms, consistent tendencies were not observed between VOC exposure, symptoms, and HF.

5. Conclusions

In-situ real-time monitoring of VOC exposure and HRV were conducted for eight MCS patients using a VOC monitor, a Holter monitor, and a time-activity pattern for 24 h to identify the relationship between VOC exposure, biological effects, and subjective symptoms of MCS patients in actual life. The results showed that there were no significantly different parameters for averaged values such as VOC exposure concentration, HF, and LF/HF compared with previous data from healthy subjects. Between HF and VOC change amount, significant negative correlations for four out of eight subjects were observed. These results suggest that some patients show inhibition of parasympathetic activities along with VOC exposure as seen with healthy subjects in a previous study. Comparing the parameters during subjective symptoms and normal conditions, VOC concentrations and/or VOC change amounts were high in all subjects except for one, and the values of HF were low for five subjects during subjective symptoms, suggesting the presence of high VOC concentration or change and low HF power when the subjects feel symptoms. Examining the time-series data for VOC exposure and $\log_{10}$ HF of each subject revealed subjects whose subjective symptoms, VOC exposure, and HF seemed well related and other subjects whose findings did not appear related. Though there are limitations of the study design and the method, characteristics of relationship between exposure and symptoms were suggestive, and based on these characteristics, prevention measures of symptoms for each subject may be proposed.

Acknowledgments

We thank Takako Matsui (Akita University Graduate School of Medicine) and Manabu Ozawa (Kitasato University Kitasato Institute Hospital). We are grateful to all the subjects who participated in this study. This study was supported by Health Labour Sciences Research Grant and Grant-in-Aid for Scientific Research (A) 19201007. Part of this research was conducted by Tokyo Metropolitan Collaboration of Regional Entities for the Advancement of Technological Excellence, Japan Science and Technology Agency (JST). This study was also supported by a health science research grant from the Japan Ministry of the Environment.

Author Contributions

Kazukiyo Kumagai, Naomichi Yamamoto, and Miyuki Noguchi were responsible for the VOC measurements and analysis. Kazuhiro Yoshiuchi and Hiroaki Kumano were responsible for HRV measurement and analysis. Kou Sakabe was responsible for subject selection and recruitment. Yukio Yanagisawa had the original idea for the study. Atsushi Mizukoshi wrote this paper. All the authors contributed to the draft of the manuscript, read, and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; and in the decision to publish the results.

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Environ Health Prev Med (2015) 20:185–194
DOI 10.1007/s12199-015-0448-4

REGULAR ARTICLE

Assessment of cerebral blood flow in patients with multiple chemical sensitivity using near-infrared spectroscopy—recovery after olfactory stimulation: a case–control study

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Received: 26 December 2014 / Accepted: 4 February 2015 / Published online: 15 February 2015

Abstract
Objectives Multiple chemical sensitivity (MCS) is a chronic acquired disorder characterized by non-specific symptoms in multiple organ systems associated with exposure to odorous chemicals. We previously observed significant activations in the prefrontal cortex (PFC) during olfactory stimulation using several different odorants in patients with MCS by near-infrared spectroscopy (NIRS) imaging. We also observed that the patients with MCS did not adequately distinguish non-odorant in the late stage of the repeated olfactory stimulation test. The sensory recovery of the olfactory system in the patients with MCS may process odors differently from healthy subjects after olfactory stimulation.
Methods We examined the recovery process of regional cerebral blood flow (rCBF) after olfactory stimulation in patients with MCS. NIRS imaging was performed in 6 patients with MCS and in 6 controls. The olfactory stimulation test was continuously repeated 10 times. The study also included a subjective assessment of the physical and psychological status and of the perception of irritating and hedonic odors.

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Results After olfactory stimulation, significant activations were observed in the PFC of patients with MCS on both the right and left sides compared with controls. The activations were specifically strong in the orbitofrontal cortex (OFC). Compared with controls, autonomic perception and feelings identification were poorer in patients with MCS. OFC is associated with stimuli response and the representation of preferences.
Conclusions These results suggest that a past strong exposure to hazardous chemicals activates the PFC during olfactory stimuli in patients with MCS, and a strong activation in the OFC remains after the stimuli.

Keywords Cerebral blood flow · Multiple chemical sensitivity · Near-infrared spectroscopy · Olfactory stimulation · Orbitofrontal cortex · Recovery

Introduction

Multiple chemical sensitivity (MCS) is a chronic acquired disorder characterized by non-specific and recurrent multisystem symptoms associated with exposure to common odorous chemicals such as organic solvents, pesticides, cleaning products, perfumes, environmental tobacco smoke, or combustion products [1–3]. According to population-based surveys, the prevalence of MCS is estimated to range from 8 to 33 % [4–9]. Thus, MCS has become a large public health concern during the past two decades, particularly in industrialized countries. The symptoms of MCS can be mild to disabling, and they are triggered by multiple chemicals. These symptoms are reactions to previous chemical exposure that recur on subsequent exposure to the same or structurally unrelated chemicals at levels below those established as having harmful effects in the general population [2]. Central nervous system (CNS) symptoms such as headaches, dizziness, extreme fatigue, and concentration difficulties are common; airway and gastro-intestinal tract symptoms are also frequently reported [2, 10–12]. Diagnosis of MCS can be difficult because of the inability to assess the causal relation between exposure and symptoms [3, 13]. No standardized objective measures to identify MCS and no precise definition of this disorder have been established. Most definitions of MCS are qualitative, relying on subjective reports from patients and clinicians of distressing symptoms and environmental exposure [14].

We previously conducted a near-infrared spectroscopy (NIRS) activation study on olfactory stimulation in patients with MCS [14]. Activation was defined as a significant increase in the regional cerebral blood flow (rCBF) following odorant stimulation. Changes in the blood flow and oxygenation to the brain are closely linked to neural activity [15]. NIRS has been commonly applied in studies of prefrontal activity [16, 17] and is suitable for detecting oxygenation changes in higher cortical regions. Our previous study identified acute activation in the prefrontal cortex (PFC) during olfactory stimulation with several different odorants in patients with MCS [14]. The prefrontal area connects to the anterior cingulate cortex (ACC), an area of odorant-related activation in patients with MCS [18]. The results of challenge tests by exposure to odorous chemicals indicated a neuro-cognitive impairment in patients with MCS, and using single photon-emission computed tomography, brain dysfunction was found particularly in odor-processing areas, thereby suggesting a neurogenic origin of MCS [19]. One possibility is that patients with MCS may have an enhanced top–down regulation of odor response via the cingulate cortex. These findings also suggest that prefrontal information processing associated with the odor-processing neuronal circuits and memory from a past experience of chemical exposure may play significant roles in the pathology of this disorder.

Our previous study also showed that the patients with MCS adequately distinguished non-odorant in 10 odor repetitions during the early stage but not in the late stage of the olfactory stimulation test when the olfactory stimulation test was continuously repeated 10 times. Repeated or prolonged exposure to an odorant typically leads to a stimulus-specific decrease in olfactory sensitivity to that odorant, but sensitivity recovers over time in the absence of further exposure [20]. Thus, we postulate that prefrontal information processing in patients with MCS is activated by an emotional response to a repeated olfactory stimulation in the late stage of the test, and that the processing system in the PFC cannot respond adequately. Further, the sensory recovery of the olfactory system in patients with MCS may process odors differently from healthy subjects after olfactory stimulation. Although recovery is generally evident after short olfactory stimulation on the several tens of second time scale [21, 22], the recovery process of patients with MCS may differ from that of healthy subjects. In this study, we examined the recovery process after short olfactory stimulation in patients with MCS, using NIRS imaging.

Methods

Patients

Patients with MCS were diagnosed in the outpatient department for people with chemical sensitivities in the Hyakumanben Clinic (Outpatient Department of Sick House Syndrome) between October 2009 and January 2013. The same case definitions for MCS (inclusion and

exclusion criteria), as in our previous study [14], were applied in this study. MCS was diagnosed according to the 1999 consensus criteria [23]. Patients diagnosed with chronic fatigue syndrome, fibromyalgia syndrome, or mental health disorders were excluded from the study. Patients who had hyperpiesia, hyperlipidemia, diabetes, and/or allergic rhinitis were also excluded. Recruitment for this study was conducted 3 months prior to the olfactory stimulation test using NIRS. The MCS condition of all patients was confirmed by the clinic physician during recruitment. Controls were recruited and selected to match the patients by age and sex at the group level. The same inclusion and exclusion criteria were applied for all patients and controls as those in our previous study [14]. Inclusion was based on the scores of the Quick Environmental Exposure and Sensitivity Inventory (QEESI), whereas exclusion criteria included abnormal hematological examinations, smoking, drug or alcohol abuse, medications, pregnancy, and severe nasal stuffiness.

This study was approved by the ethical committee for human research at the Hyakumanben Clinic (99642-61) and the Louis Pasteur Centre for Medical Research (LPC.11) and was performed according to the guidelines of the Declaration of Helsinki (1975). All patients provided written informed consent and received the equivalent of 5000 JPY for their participation. This study was conducted from November 2012 to March 2013.

Olfactory stimulation

The same card-type olfactory identification test kit (Open Essence; Wako Pure Chemical Industries, Ltd., Osaka, Japan) and odorants [mandarin orange (MO), Japanese cypress (JC), menthol (Mt), and perfume (Pf)] as those in our previous study were used for the olfactory stimulation test [14]. Perception of these odors was tested by placing the card at approximately 30 mm from the noses of both patients with MCS and controls.

Experimental procedure

In the present study, we followed the same experimental procedure as that in our previous study [14] and added a recovery period after the olfactory stimuli. Interviews were conducted immediately before the olfactory stimulation test and the assessments of health and nasal symptoms. The test room was maintained at a temperature of approximately 23 °C. Patients sat in a comfortable chair and remained in the test room long enough to feel comfortable before being exposed to the odorants. During the experiments, the patients closed their eyes and slowly repeated the Japanese alphabet in an undertone to establish a stable rCBF prior to the olfactory stimulation. They stopped repeating the Japanese alphabet and closed their eyes during the olfactory stimulation, which lasted for 10 s. Olfactory stimulation was performed after a 30-s pre-rest period to establish the baseline level (Fig. 1).

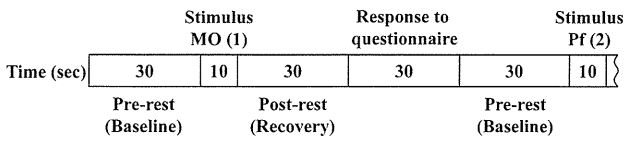

Fig. 1 Experimental protocol. First, the subjects had a 30-s pre-rest. Then, the subjects were given an olfactory stimulus for 10 s, followed by a 30-s post-rest and 30-s self-reporting to a questionnaire on irritating and hedonic scales, respectively. The 100-s cycles per odorant were repeated 10 times in a row

The questionnaire on irritating and hedonic scales was completed immediately after a 30-s rest period (post-rest) to allow recovery after the olfactory stimulation. The response time for the questionnaire was secured for 30 s. After that, the same process was repeated for an additional 9 olfactory stimuli. Irritation was evaluated on a visual analogue scale, with responses ranging from "not at all" to "strong". Hedonic responses were rated on a 5-point Likert scale ranging from pleasant (1) to unpleasant (5).

Olfactory stimuli were applied in the following order: MO, Pf, non-odorant (NO), JC, Mt, Pf, JC, NO, Mt, and MO. The order of the first block was as follows: MO (1), Pf (2), NO (3), JC (4), and Mt (5). The order of the second block was permuted from the first block as follows: Pf (6), JC (7), NO (8), Mt (9), and MO (10). The 100-s cycles were repeated 10 times in a row. Thus, the order of 10 repetitions (1–10) was as follows: MO (1), Pf (2), NO (3), JC (4), Mt (5), Pf (6), JC (7), NO (8), Mt (9), and MO (10).

NIRS data acquisition

NIRS works on the principle that near-infrared light is absorbed by oxygenated (oxyHb) and deoxygenated (deoxyHb) hemoglobin (Hb) but not by other tissues. Changes in oxyHb concentration in the PFC were measured using the functional NIRS topography system OMM-3000 Optical Multi-channel Monitor (Shimadzu Corporation, Kyoto, Japan). These changes reflect neuronal activity as their levels correlate with evoked changes in rCBF [15, 24, 25]. When neurons become active, local blood flow to the relevant brain regions increases and oxygenated blood displaces deoxygenated blood. Measurement of oxyHb concentrations is most useful because changes in oxyHb are the most sensitive indicators of changes in rCBF among the three NIRS parameters (oxyHb, deoxyHb, and totalHb) [26, 27]. Pairs of illuminators and detectors were set 3 cm apart in a 3 × 9 lattice pattern to form 42 channels through a holder set in the PFC. Changes in the oxyHb concentration were recorded every 130 ms using the NIRS

system. Optical data were analyzed on the basis of the modified Beer–Lambert Law and signals reflecting the oxyHb concentration changes in an arbitrary unit were calculated (millimolar–millimeter) [14].

Questionnaire on physical and psychological status

Patients completed a self-report questionnaire for the assessment of physical and psychological parameters, which included the Chemical Sensitivity Scale for Sensory Hyper-reactivity (CSS-SHR) [28], the Somato-Sensory Amplification Scale (SSAS) [29], the Autonomic Perception Questionnaire (APQ) [30], the Tellegen Absorption Scale (TAS) [31], the Marlowe–Crowne Social Desirability Scale [32], the Taylor Manifest Anxiety Scale (TMAS) [33], the Negative Affectivity Scale (NAS) [34], and the Toronto Alexithymia Scale (TAS-20) that evaluates the total score and the scores of the three subscales, which assess difficulties in identifying feelings (DIF), difficulties in describing feelings (DDF), and externally-oriented thinking (EOT) [35].

Statistical analyses

To assess the recovery status after the olfactory stimulation, the oxyHb concentrations between the 30-s rest period after the olfactory stimulation and the baseline during the pre-rest period were compared in each channel. Because raw data of NIRS provided only relative values and could not be averaged directly across patients or compared among channels, raw data from each channel were converted into z-scores [14, 36–38]. In the present study, we used the same statistical analyses as that in our previous study [14]. The t test was used to compare the brain activity obtained from NIRS imaging for each channel between cases and controls. The non-parametric Mann–Whitney U test was used to analyze the results of the olfactory stimulation questionnaire and to quantify the differences between patients with MCS and controls. The t test was applied to analyze the results of the physical and psychological scales to determine differences between patients with MCS and controls at baseline. All data analyses were performed using the SPSS statistics software, version 22.

Results

Participants

Participants included 10 patients with MCS (age, 28–64 years; mean, 51.0 ± 10.6 years; all females) and six controls (age, 36–58 years; mean, 45.7 ± 8.3 years; all females). Three patients with MCS did not fulfill the inclusion criteria of QEESI. The fourth patient with MCS had severe allergenic reactions to allergens of cedar pollen, mites, cats, dogs, and fungi, and showed a high value of immunoglobulin E type on hematological examinations. The remaining six non-smoking patients with MCS (age, 49–64 years; mean, 54.5 ± 5.9 years; all females) and six non-smoking controls (age, 36–58 years; mean, 45.7 ± 8.3 years; all females) passed all criteria and were included in the analyses. All six patients with MCS had participated in our previous study [14]. Of the six controls, two controls had participated in our previous study [14] and the remaining four controls were participating for the first time in the present study. All patients with MCS tried to avoid the exposure to odorous chemicals as much as possible. These patients were homemakers or pensioners and their occupational histories showed that previous occupations included a clerical employee (office or retail store), a fabric tinter, and a supermarket baker. Two controls also tried to avoid exposure to odorous chemicals as much as possible. Their occupations were teacher. Four controls did not consciously try to avoid exposure to odorous chemicals, and their occupations were as follows: teacher, office worker, child welfare volunteer, and the fourth was a homemaker whose previous occupation was in sales for a general insurance company.

NIRS imaging and subjective evaluation of odors

Time-course of average z-scores of all channels for oxyHb in the patients with MCS group and controls during pre-rest, stimulus, and post-rest are shown in Fig. 2. Results of the t test in terms of the average of all channels (1–42) comparing z-scores for oxyHb concentrations between patients with MCS and controls are shown in Table 1. The first olfactory stimulation with MO (1) lead to increased rCBF levels in the PFC, which was not significantly different between patients with MCS and controls.

Increases in rCBF levels in patients with MCS were suppressed after exposure to the non-odorant tests NO (3) and NO (8) on the third repetition. There was no difference between patients with MCS and controls in the PFC responses. Significant differences in the PFC responses were observed between patients with MCS and controls after Pf (2), JC (4), Mt (5), Pf (6), Mt (9), and MO (10) olfactory stimuli. The increases in the PFC response after the olfactory stimulation were significant in patients with MCS. No significant difference was observed in the JC (7) test, but as shown in Fig. 2, MCS patients had increases in rCBF levels during 10 s after the stimulus, which were not observed in the control group.

Table 2 shows the correlation coefficient between rCBF after the first and second exposure to the same odor in terms of z-scores for all channels (1–42). Comparing the

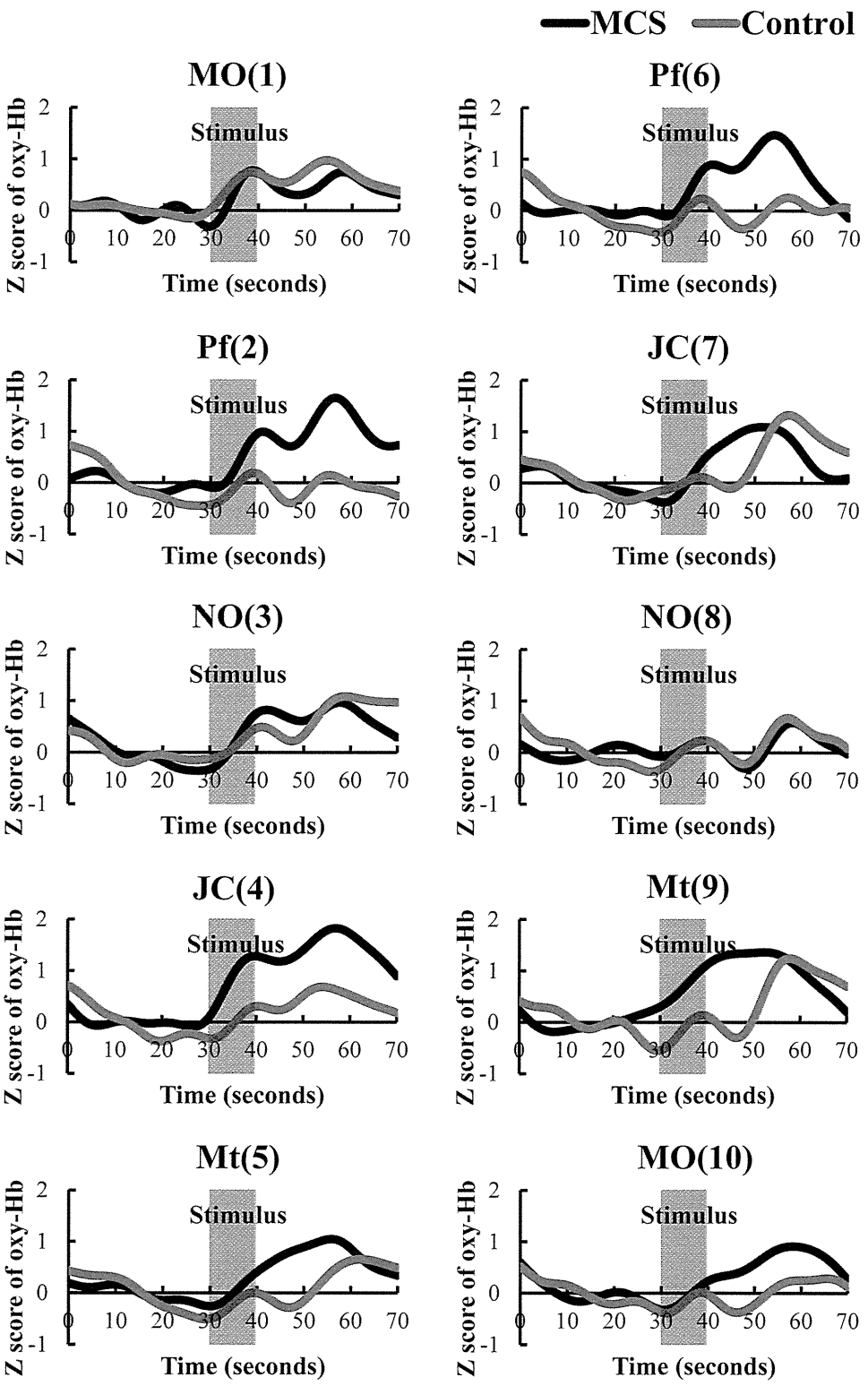

Fig. 2 Time-course of average *z*-scores of all channels for oxyHb in patients with MCS ($n = 6$) and controls ($n = 6$) during pre-rest (baseline, 10–30 s), stimulus (30–40 s), and post-rest (recovery, 40–70 s). *Y*- and *X*-axes represent *z*-scored oxyHb values and times. Law and signals reflecting the oxyHb concentration changes in an arbitrary unit were calculated (millimolar–millimeter). Data of the signal were adjusted by an FFT (Fast Fourier Transform) filter smoothing technique (OriginPro 9.1 software of OriginLab Corporation). The cutoff frequency was determined at thirty-five points. MCS group is indicated as a *black line* and control is indicated as *gray line*. *MO* mandarin orange, *Pf* perfume, *NO* non-odorant, *JC* Japanese cypress, *Mt* menthol. *Numbers in parentheses* indicate the orders of the 10 repetitions (1–10)

rCBF between the first and second exposures revealed significant correlations in both patients with MCS and controls for all stimuli, with the exception of MO and NO in patients with MCS. The correlation coefficients of patients with MCS were lower overall than those of controls. As in our previous study [14], the variation within the MCS group was larger than in the control group. In the subjective evaluation, both patients with MCS and controls

Table 1 The *t* test results in terms of average values for all channels (1–42) comparing *z*-scores for oxyHb between patients with MCS and controls

Test	MCS ($n = 6$)	Controls ($n = 6$)	*p* value
MO (1)	0.47 (2.40)	0.66 (2.05)	0.336
Pf (2)	1.05 (2.57)	−0.11 (1.33)	<0.001*
NO (3)	0.70 (2.47)	0.73 (1.48)	0.895
JC (4)	1.41 (3.37)	0.40 (1.35)	<0.001*
Mt (5)	0.73 (3.02)	0.23 (1.08)	0.013*
Pf (6)	0.82 (3.13)	−0.03 (1.02)	<0.001*
JC (7)	0.66 (3.11)	0.63 (2.63)	0.905
NO (8)	0.13 (1.15)	0.23 (1.34)	0.407
Mt (9)	1.06 (2.93)	0.56 (2.50)	0.038*
MO (10)	0.61 (1.59)	0.01 (0.94)	<0.001*

Values are expressed as means (±standard deviations). Numbers in parentheses in column 1 indicate the order of the 10 repetitions (1–10)

MO mandarin orange, *Pf* perfume, *NO* non-odorant, *JC* Japanese cypress, *Mt* menthol

* Significant at $p < 0.05$

Table 2 Correlation coefficient (*r*) between rCBF after the first and second exposures to the odor in terms of *z*-scores for all channels (1–42)

Odorant	MCS ($n = 6$)		Controls ($n = 6$)	
	r	*p* value	*r*	*p* value
MO	0.082	0.197	0.193	0.002*
Pf	0.321	<0.001*	0.548	<0.001*
NO	0.092	0.144	0.447	<0.001*
JC	0.250	<0.001*	0.479	<0.001*
Mt	0.302	<0.001*	0.400	<0.001*

Values are expressed as Pearson product-moment correlation coefficients

MO mandarin orange, *Pf* perfume, *NO* non-odorant, *JC* Japanese cypress, *Mt* menthol

* Significant at $p < 0.05$

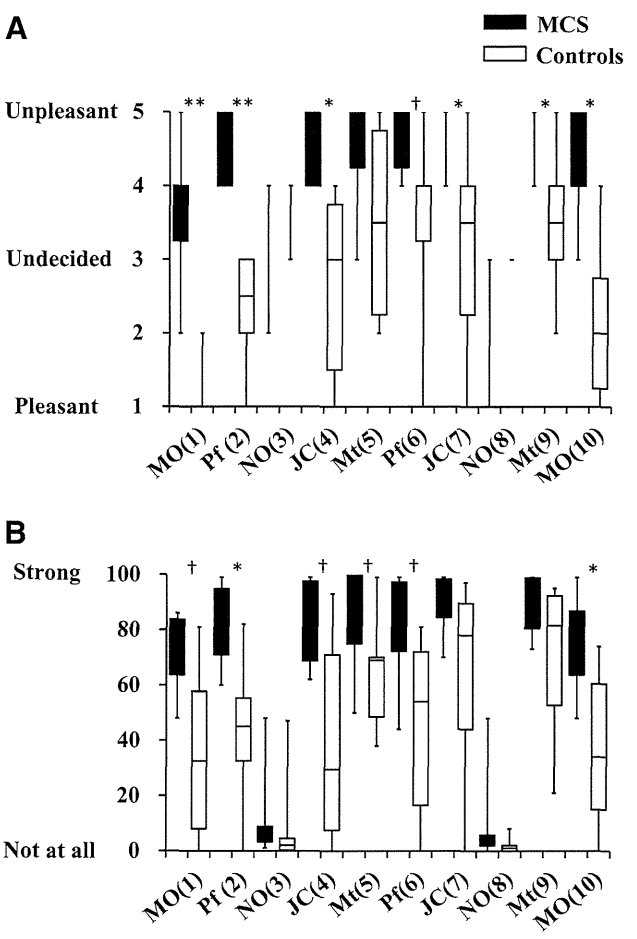

Fig. 3 Ratings of hedonic (**a**) and irritating (**b**) odors by patients with MCS ($n = 6$) and controls ($n = 6$) after the olfactory stimulation. *MO* mandarin orange, *Pf* perfume, *NO* non-odorant, *JC* Japanese cypress, *Mt* menthol. *Numbers in parentheses* indicate orders of the 10 repetitions (1 to 10). Statistically significant differences between groups are indicated. $*p < 0.05$, $**p < 0.01$. Significant tendencies are indicated: $^{\dagger}p < 0.10$

responded "not at all" on the irritation scale and "undecided" on the hedonic scale for NO (Fig. 3). The results of the hedonic scale indicated that scores of most patients with MCS were significantly higher than those of controls. The results of the irritation scale indicated that the Pf (2) and MO (10) scores of patients with MCS were significantly higher than those of controls. Scores for MO, Pf, JC, and Mt were also higher in the patients with MCS than in controls, but the differences were not statistically significant. Large ranges of scores in patients with MCS and controls were assumed to be causally related to the results.

Figure 4 provides the topographical maps of average *z*-scores for oxyHb in patients with MCS and controls. Figure 5 shows the average *t* values for each channel comparing the *z*-scores for oxyHb between patients with MCS and controls. Even after olfactory stimuli, significant activations were observed in the PFC of patients with MCS on both right and left sides (distinct from the center of the PFC) compared with controls. Activation was defined as a significant increase in rCBF due to olfactory stimulation. The activations were especially strong in the lateral orbitofrontal cortex (OFC), on both the right and left sides of the OFC in the PFC (Fig. 4). These remaining activations after the olfactory stimuli were stronger in the test for Pf (2) on the second repetition, for JC (4) on the fourth repetition, for Mt (5) on the fifth repetition, and for Pf (6) on the sixth repetition. After the test for Pf (6), the regional differences of the activation area between patients with MCS and controls were decreased. Our previous study suggested that the olfactory system in patients with MCS could not adequately process odors in the late stage of the