An experimental module for olfactory fMRI experiments

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Abstract: Clinical examination for olfactory-associated diseases is a problematic issue and is often interfered by the objective and subjective factors. Functional magnetic resonance imaging (fMRI) that provides information for odor-induced brain activation can be used as an important basis for the assessment in the olfactory-associated diseases and cognitive science. In this study, we used the homemade continuous positive airway pressure (CPAP) to give olfactory stimuli. In early experiments, smoking tests were used to prove that the odor molecules can be removed in a fixed time difference. The preliminary results showed that visual smoke at the flow rate of 1~10ml/s could arrive to the end of the terminal within 1 second after the valve was opened. In this case, the flow of experimental procedures can be precisely controlled. Then we acquired the data of blood-oxygen level dependent brain fMRI (BOLD-fMRI) of ten healthy subjects. The results showed that the average of activated voxels are 56±23 ($P<0.001$) in the primary olfactory cortex (piriform cortex) and 97±36 ($P<0.001$) in the prefrontal secondary olfactory cortex (orbitofrontal cortex), respectively. In the brain activation regions, the experiment module was shown that could effectively detect olfactory response and be valuable to the clinical diagnosis.
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1. Introduction

Only a few studies on olfaction have been done in the past, however, research in this field is gradually getting attention. Studies have been done not only in behavioral medicine, but also in cognitive science and genomics. For example, the studies published in the internationally renowned journal "Cell" by Richard Axel and Linda Buck, 2004 Nobel in Physiology and Medicine laureate, found that about 1,000 olfactory genes in human body (about 3% of the total genes in human) [1], which showed the importance and complexity of this field. Recently studies on olfaction also connected with market economics and developed a new term - olfactory marketing (scent marketing) [2-4]. Because human's olfaction is directly connected with the memory and emotion center of the brain, the smell will directly affect the emotions, and emotion will directly affect the action [4]. Also, human's olfactory system cannot be shut down or stop (holding the breath is just a temporary gripe). Under this assumption, a corporate marketing strategy has designed. Hence it is shown that the olfaction has significant effects on human's conscience and action. We need an appropriate tool which could quantify the olfactory function.

There are several olfactory testing methods for clinical use such as olfactory threshold, olfactory discrimination and olfactory identification [5-8]. However, it has not yet had a well-accepted standard. The otolaryngologists usually used Sniffin'Sticks test (including these three testing methods) whose function is quite complete and being used widely in clinical use to diagnose whether patients have olfactory disorders [9]. But Sniffin' Sticks olfactory test needs to rely on the patient's subjective awareness to judge and answer the questions, hence it may produce incorrect results for patients with awareness disorder or some subjects that either are unfit or have special attempts.

Some studies use functional magnetic resonance imaging (fMRI) to investigate the reaction of human brain to flavor and odor stimuli [10-13]. The advantage of using fMRI is that the subjects receive no radiation exposure and these tests are not aggressive, but effective to find out the reactions of the cerebral cortex to sensory stimulation. Olfactory receptor is a chemoreceptor which is different with the photoreceptors of the eye and the mechanoreceptors of the ears. The binding of odor molecules to olfactory receptor leads to the conducting to the olfactory bulbs via the olfactory nerve and then transmitting to the

olfactory cortex. Using fMRI, it is mainly observed the reaction in the primary olfactory cortex (piriform cortex) and secondary olfactory cortex (orbitofrontal cortex). These two anatomical locations are important places that produce a reaction and olfactory discrimination when the odor enters the brain. Koizuka and Sobel tried to demonstrate that BOLD-fMRI responded in primary olfactory cortex but did not get good results due to the constraints of the instruments and statistical methods [10-12]. Later Cerf-Ducastel improved the experimental design and used the olfactory fMRI to confirm the phenomenon that the dysosmia of old people is due to the degradation in the primary olfactory cortex [13].

Olfactory fMRI often requires apparatus with complex feedback and is not easy to set up for clinical use. Our study improved the design of Poellinger [14] and Popp *et. al.* [15] and used the pipe and material available in clinical units to test the handling and reproducibility of the improved design, hoping to provide the clinical units an affordable, simple and reliable fMRI olfactometer for further olfactory experiments.

2. Materials and Methods

2.1 Continuous positive airway pressure device

This study used continuous positive airway pressure (CPAP) device to provide the main gas stream. The positive air pressure unit (to push the flavored molecules to enter the detected side) and negative pressure suction unit (to take away the flavored molecules away from the detected end) are from central medical gas supply system in the MRI examination room.

The CPAP unit could control the flow rate with the flow-meter in the range of 1 to 10 mL/s. Using a three-way stopcock to switch experimental odor and normal air. The source of odor was banana oil (0.1 ml ethyl acetate + 50ml 95℃ water) added in 2000mL large erlenmeyer flask and connected with two sections of glass tubes (one for gas in and the other for gas out). After connecting, using Teflon pipeline to connect flow-meter, three-way stopcock, flasks and the detected side of head coil (within 5cm inside the nostril of subjects). Teflon pipeline could prevent the odor on the pipeline. The detected side coupled with a negative pressure system (-240mmHg) can take away the smell and avoid residual odor affect the experiments correctness (Figure 1).

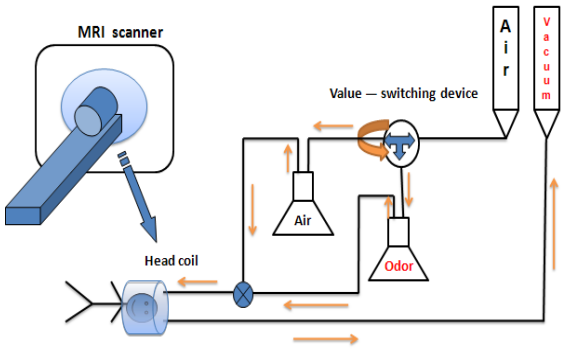

Figure 1: The pipeline design of olfactory experiment.

2.2 Preliminary tests of visualized smoke flow-rate

After CPAP device was set up, first burned incense to produce the fume in the erlenmeyer flask. When the flask was filled with white fume, the flask was sealed. The camera and bright flashlight were erected at the end of Teflon tube. Then adjusted the flow meter from 1 to 10 mL/s, and recorded the continuous video of the three-way stopcock from the opening of the tube to 10 seconds, then the smoke emitted into the negative pressure system, sucked and forcefully expelled from the tube. The video was taking 3 times for each flow rate. Lastly checked the video and recorded the time difference between the time that the valve was opening 10 seconds and the time that the smoke was fully ejected (Figure 2).

Figure 2: The preliminary results of tests of visualized smoke flow-rate: (A) first burned incense to produce the fume in the erlenmeyer flask for observation (B) At 1mL/s, the shape of visualized smoke was in a columnar shape. (C) At 10 mL/s, the visualized smoke was sprayed.

2.3 Volunteer subjects

10 subjects (5 male and 5 female); the range of age is from 18 to 22 years old. All subjects had normal olfactory function and filled out a questionnaire for the

record of medical history and specific situation. The following cases were excluded: 1. a history of sinusitis or asthma. 2. infection in the upper respiratory tract in two weeks before the test. 3. dysosmia. 4. nasal polyps or nasopharyngeal cancer. 5. intracranial lesions. 6. smoking. 7. olfactory injury caused by accidents. 8. Equipped with items that could not be removed and nearby high magnetic field (such as heart rhythm instruments). 9. Equipped with metal dentures. 10. claustrophobia. 11. unwilling to participate in the tests. The study was approved by institutional review board (IRB) (CSMUH No: CS08112) of the Chung Shan Medical University Hospital and all subjects had filled out the consents.

2.4 BOLD-fMRI

The setup was to use a 1.5 Tesla Siemens MAGNETOM Sonata (Erlangen, Germany) MRI.

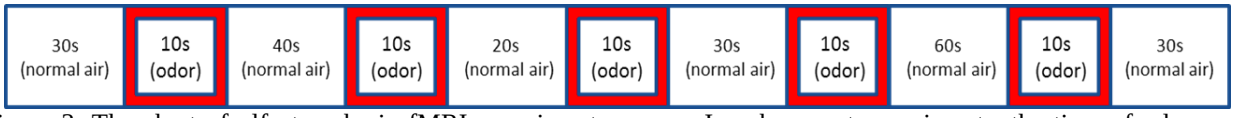

Figure 3: The chart of olfactory brain fMRI experiment process. In subsequent experiments, the time of odor were prolonged to 16s, 20s.

Scanner and standard fMRI experiment pulse sequence (echo-planar imaging pulse sequence: TR: 2000 ms; TE: 75 ms; Matrix Number: 128×128; FOV: 200× 200 mm; Slice Thickness: 5 mm; Total Slice: 25) to obtain the BOLD-fMRI data. The olfactory brain fMRI experiment: the subjects were in blinded experiment. Each time opened the tube and let odor stimulus spread for 10 seconds (the odor of ethyl acetate) with airflow rate of 8 mL/s, and added the background state in the intervals of unequal-length (respectively 40 seconds, 20 seconds, 30 seconds, 60 seconds, 30 seconds of normal air). In total there were 5 times of simulation cycles. Each inspection began from 30 seconds background state and ended with 30 seconds background state. The experimental process was shown in Figure 3.

The time for ethyl acetate odor spread was then changed from 10 seconds to 16 seconds and 20 seconds respectively, and other steps were the same, i.e. added the ground state in the intervals of unequal-length (respectively 40 seconds, 20 seconds, 30 seconds, 60 seconds, 30 seconds of air) between stimuli.

2.5 Data analysis

We used SPM2 (Statistical Parametric Mapping 2；By members & collaborators of the Wellcome Trust Centre for Neuroimaging) for statistical analysis of fMRI data. In addition to the standard SPM analysis process, we did some partial sampling analysis: in the data of stimulating 10 seconds with the odor, we took the first three TR images from 5 TR images taken in 10 seconds and removed the data taken in the latter 4 seconds. Similarly, the data of stimulating 16 and 20 seconds also processed in the same manner before SPM analysis.

3. Results

The results of tests of visualized smoke flow-rate are shown in Table 1. The result showed that the smoke can arrive to the end of the tube at the air flow rate of 1mL/s~10mL/s in 1 second. The difference due to different velocity was mainly in the form of the airflow when the visualized smoke spread. At 1mL/s, the shape of the visualized smoke was in a columnar shape (Fig. 2B) while at 10 mL/s, the visualized smoke was sprayed (Fig. 2C). But in either case, negative pressure systems could take away the smoke in 1.5 seconds.

Then we used VOLUME CLUSTER (built in SPM2 software) and found the average number of activated voxels in the secondary olfactory cortex. Six of ten subjects showed activation in the right side secondary olfactory cortex were greater than the left side. (figure 4.a and 4.b) The activation in primary olfactory cortex (piriform cortex) of the temporal lobe were shown only in five subjects (figure 4.c) and the average number of activated voxels was 28±13 on the right side and 19±15 on the left side ($P < 0.001$) (Table 2).

In the experiment with 16 seconds of odor stimuli, the activation was found in the orbitofrontal cortex and the middle frontal gyrus of six subjects and the average number of activated voxels was 37±16 ($P < 0.001$). And the activation was found in the temporal lobe of the primary olfactory cortex of only one subject and the number of the activated voxels was 41 ($P < 0.001$). And in the experiment with 20 seconds of odor stimuli, the activation was found in the orbitofrontal cortex of three subjects and the average number of activated voxels was 31±14 ($P < 0.001$) but no sign of activation was found in the olfactory cortex.

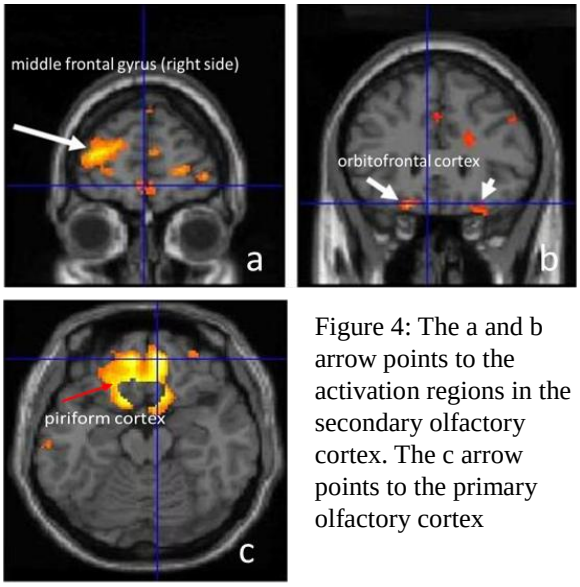

Figure 4: The a and b arrow points to the activation regions in the secondary olfactory cortex. The c arrow points to the primary olfactory cortex

the first 6 seconds sample analysis showed activation in the orbitofrontal cortex of all 10 subjects and the average number of activated voxels was 50±28 (P <0.001). The activation was also found in the temporal lobe of the primary olfactory cortex of eight subjects and the number of the activated voxels was 34±15(P <0.001). In the experiment with 16 seconds of odor stimuli, the first 6 seconds sample analysis showed activation in the orbitofrontal cortex of 9 subjects and temporal lobe of the primary olfactory cortex of six subjects and the number of the activated voxels was 32±10（P<0.001）. In the experiment with 20 seconds of odor stimuli, the first 6 seconds sample analysis showed activation in the orbitofrontal cortex of 7 subjects and the average number of activated voxels was 30±16 (P < 0.001). The activation was also found in the temporal lobe of the primary olfactory cortex of five subjects and the number of the activated voxels was 28±11（P<0.001）.

In the experiment with 10 seconds of odor stimuli,

TABLE 1. The time of arriving to the end of the tube and removing the smoke at different velocities

Airflow velocities	1mL/s	2mL/s	3mL/s	4mL/s	5mL/s	6mL/s	7mL/s	8mL/s	9mL/s	10mL/s
Smoke arrived	0.95	0.90	0.90	0.85	0.85	0.85	0.80	0.80	0.80	0.75
Smoke removed#	0.90	0.95	0.95	1.05	1.10	1.20	1.25	1.25	1.30	1.45

To remove the smoke, the suction device adopts negative pressure 240mmH_2O. After the smoke is closed, the time that needs to remove the smoke is recorded in seconds.

TABLE 2. The coordinate and volume of olfactory activation region of brain

Activation region	left/right	coordinate (mm)			Activation voxels	z-values
		X	Y	Z		
primary olfactory cortex	Right	-21 ± 3	5 ± 5	-18 ± 3	28±13	3.51
	Left	23 ± 4	3 ± 7	-19 ± 5	19±15	1.53
orbitofrontal cortex	Right	-25 ± 6	39 ± 4	-17 ± 4	56±23	2.59
	Left	27 ± 5	37 ± 6	-20 ± 5	25±18	1.84
middle frontal gyrus	Right	-41 ± 7	41 ± 4	7 ± 9	72±21	2.78
	Left	46 ± 5	40 ± 9	9 ± 5	36±28	1.52

4. Discussion

This study improved the principle of making respirators used by subjects in the paper of Popp *et. al.* [15], whose main feature is that uses positive airway pressure system which is available in general clinical examination room to transport smell and also connects to a negative pressure pipeline to pull out the odor and avoids residual odor to have effects on the experimental accuracy.

In addition, we also take reference from Poellinger and Popp *et. al.* [14-15] and modified the design into an open and stable CPAP device. This improvement could avoid the sense of oppression of subjects when wearing a mask and the discomfort due to the gas blowing directly in the subject's face. Hence the subjects could smell the odor and avoid irritable mood in the most natural and comfortable state. We tried to design a simple and low-cost MRI apparatus and combined with fMRI experiment with olfactory stimuli for direct measurements of the stimulus and response sequence on the cerebral cortex to further establish a simple operable and reliable experimental module which could be used repeatedly in various clinical tests for dysosmia.

The continuous image obtained in the tests of visualized smoke flow-rate showed that the smoke can be sent to the end of the tube in time in the gas flow rate

of 1~10 mL/s and this could demonstrate the accuracy of controlling time sequence when gas molecules were in and out scanning the images and exclude the possibility of residual odor. Each flow rate test was repeated three times to confirm that it could show experimental reproducibility stably in this system.

A continuously repeating section was basic and commonly-used for fMRI experiments such as the rotation between the rest state and the activation status that invokes by additional stimulus, e.g., flash, voice and finger movement, etc. This feature was suitable for SPM statistical model and had the advantage that had a higher contrast to noise ratio (contrast to noise ratio, CNR) in the BOLD signal. It was useful for the detection of non-cognitive brain function experiments but the drawback was vulnerable due to adaptation or fatigue. These problems occurred easily in the olfactory experiment; this study changed the design to the random and longer interval and tried to avoid the occurrence of olfactory fatigue.

The results also confirmed that the gas supply system could be applied in the tests of olfactory response. In the experiments with 16 seconds and 20 seconds of odor stimulation, it is clearly showed that the detection rate of olfactory reaction (based on the number of the subjects that showed cortical activation reaction responded to the presence or absence of the smell, 10/10 in 10 seconds test; 6/10 in 16 seconds test; 3/10 in 20 seconds test). The number of average activated voxels in secondary olfactory cortex also declined and was consistent with the results that longer olfactory stimuli would cause sensory adaptation and subjects were gradually unable to identify the odor in the latter stimuli. We concluded that it was long enough for 10 seconds stimuli for the statistics of activation area.

The subjective feeling of the sense of smell comes from the axon of olfactory bulb that passed through the olfactory tract and ends in the outer olfactory region of the temporal lobe. The region includes the old cortex of hook portions, the cortex of entorhinal area (in front of parahippocampal gyrus), and part of the cortex of limen insulae. The hook portion, outer zone of nose and the limen insulae together are referred as the piriform cortex since animals with acute olfactory ability had a pear-shaped appearance in homology regions. Part of the amygdaloid body was also included in the lateral olfactory region; the hook portion was the markers on the surface of medial temporal lobe.

The innerback part of amygdaloid composed by the corticomedial group of nuclei could accept the olfactory fibers. It is generally believed that lateral olfactory region was the major region of olfactory stimulation responsible for cognitive awareness, the so-called “primary olfactory area”. The primary olfactory region would establish the connection with other parts of the brain (mainly in the secondary olfactory cortex - the orbitofrontal cortex) and be responsible for the emotion caused by olfactory stimuli. The detection rate of primary olfactory region is 5/10 for 10 seconds stimuli, 1/10 for 16 seconds stimuli and 0/10 for 20 seconds stimuli. This result was significantly lower than the detection rate of activation in the secondary olfactory cortex.

According to previous studies, it is because that primary olfactory cortex will tell the difference of gas molecules and passes the signal to the secondary cortex but this pattern is getting slower quickly while the sequential induction of awareness of feelings, odor memory judgment and emotional changes will last longer. In order to improve the efficiency of the detection of the primary olfactory cortex, we tried to do sample analysis of the first 6 seconds data in odor stimulation experiments, and focused on the initial response of the olfactory system when the odor molecules just entered. This could also avoid the impact of olfactory fatigue. The results showed that the detection rate of the primary olfactory cortex was 8/10 with 10 seconds stimulation, 6/10 with 16 seconds stimulation and 5/10 with 20 seconds stimulation; detection rates of secondary olfactory cortex were 10/10 with 10 seconds stimulation, 7/10 with 16 seconds stimulation and 9/10 with 20 seconds stimulation. It is apparently seen that the efficiency of detection rate of the primary olfactory cortex improved, especially of the data with longer stimulation such as 16 seconds and 20 seconds. Also, the detection rate of secondary olfactory cortex in the groups with 16 seconds and 20 seconds stimulation also increased. This implied that when odor just entered, the activation of brain cortex appeared clearly and awareness and feeling were also strong, but soon these responses would subsequently lead to sensory adaptation and the activation would be gradually invisible.

Could we reduce stimuli as short as 6 seconds in the experiment? The answer was negative. Consider the switch and operation of the gas supply system, the time is so short that the error and interference would be more obvious. Furthermore, consider the odorless part of the ground state, if the period of stimulation was too short, there might be a delay of odor or feelings when the experiment were in the period of ground state and therefore affect the results of statistical analysis. For 10 seconds stimulation, suppose that the first 6 seconds has a high peak in the activation reaction, the incidence of sensory adaptation would not affect the ground state.

5. Conclusion

In recent years, image analysis of brain function has become a popular area of medical imaging research and many radiologists had been involved in related research. In this study we built a low-cost and easy-operable olfactory experimental module but many

details need to be improved such as equipment erection, experimental processes and statistical models. These adjustable factors could be further discussed and studied to increase this module's accuracy and sensitivity. In the future, we could change the factors such as the types and number of odors, or the physiological and psychological background, or add the rhythm of odor for a new design in the experiments. Therefore, the experiment modules not only could provide clinical diagnostic information but also hope to give readers the inspiration for more and more clinical radiologists getting into this field.

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