The effect of odor exposure time on olfactory cognitive processing: An ERP study

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The present study aimed to investigate the effects of stimulus time duration on central nervous odor processing. Twenty-one young healthy males participate in our study. There are three odor mixtures in this study and every odor mixture has two different duration time (300 ms; 500 ms). The odor was presented via a computer - controlled olfactometer and EEG was recorded from 64 scalp locations. At behavioral level, the longer the odor stimulus was presented, the greater the concentration was perceived. At electrophysiological level, positive waves of 200 ms appeared in stimulation (P2), negative waves of about 300 ms appeared in stimulation (P3) components, besides, have a larger N2 amplitude than the shorter duration time condition in the mid-frontal (P3) components, besides, have a larger N2 amplitude than the shorter duration time condition in the mid-frontal and left frontal-temporal areas. These results revealed that duration time of odor mixture do have an influence on the central nervous odor processing.

Keywords
Odor mixture; oddball paradigm; olfaction; odor processing; event-related potentials

1. Introduction

Olfaction, also known as olfactics, is the sense of smell. This sense is mediated by specialized sensory cells of the nasal cavity. There are two independent chemosensory organs in the nasal cavity: olfactory epithelium and vomeronasal organ. Olfactory receptor neurons in olfactory epithelium are thought to encode different odor molecules (also known as odorant) and transmit olfactory information to the main olfactory system, which bring about olfactory experience. While vomeronasal organ encodes the pheromone and transmits the information to the accessory olfactory system, then causes the physiological and behavioral changes in animals (Firestein, 2001). There are approximately 350 olfactory receptors in the human nasal mucosa (Malnic et al., 2004), which encode different chemical structures and convert chemical information into nerve impulses.

Neuroimaging such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), have resulted in more discoveries and understanding of the brain region structure of olfactory information processing in humans (Gottfried, 2006; Hummel et al, 2005; Iamili et al, 2011; Savic at al., 2002). For example, most olfactory nerve fibers directly project to the piriform and entorhinal cortices as well as to the amygdalae, whereas a minority of fibers projects through the thalamus towards the orbito-frontal cortex (OFC) (Gottfried, 2006). One study found that right OFC is more strongly activated when subjects were judging odor familiarity than they were just trying to detect the odor (Royet and Plailly, 2004), the left OFC shows enhanced activation when subjects were evaluating odor hedonics. Using fMRI effective connectivity analysis, the connectivity between the mediodorsal thalamus and the OFC was enhanced when subjects attended to an odor as compared to attending to a simultaneously presented tone, indicating the involvement of the thalamus in conscious smell perception (Royet and Plailly, 2004). Additionally, another study indicated that both the left anterior insula and the left frontopolar gyrus are involved in odor discrimination (Royet and Plailly, 2004).

Though the fMRI and PET have a high spatial resolution, the event-related potentials (ERPs) are used to investigate odor processing in a highly time-sensitive manner, its response is directly related to the neuronal activation, which is time-locked to the stimulus onset (Rugg and Coles, 1996). The ERP study of olfactory stimulation, like visual and hearing, also has the same classical component index Negative Wave Around 100 ms Stimulated (N1), Positive waves of 200 ms or so appear in stimulation (P2), Negative waves of about 200 ms appeared in stimulation (N2), Positive waves of about 300ms appeared in stimulation (P3) and so on (see Fig. 1). N1 and P2 are two classical exogenous components that change with the stimulus. For example, the amplitude of N1 and P2 increases significantly when the stimulus concentration becomes larger (Tateyama et al., 1998; Turetsky et al., 2003). Chemosensory event-related potentials (CSERPs) can be
used in the clinical assessment of olfactory functions (Lötsch and Hummel, 2006). In clinical studies, olfactory event-related potentials (OERP) is an important diagnostic basis for mental disorders such as schizophrenia, Parkinson’s and alcohol addiction (Turetsky al., 2003; Turetsky et al., 2008; Maurage et al., 2011). Compared with healthy people, schizophrenia patients showed a significantly lower amplitude of N1 and P2 components (Kayser et al, 2010; Turetsky et al., 2003; Turetsky et al., 2008). Considering the different hedonic value of odorants (i.e., rose-like phenethyl alcohol and rotten butter-like isobutyraldehyde), people with schizophrenia have a shorter peak latency across several ERP components compared to the healthy population, and these effects were evidently most robust for N1 during the presentation of negative odors (Pause et al., 2008). Among alcoholics, electrophysiological data showed delayed olfactory N1-P2 latencies and reduced P2 amplitude (Maurage et al., 2011). In healthy adults, studies found that the OERP vary with age and gender. Compared to older adults or men, younger adults or women have generally greater amplitude and shorter latency (or men, younger adults or women have generally greater ampli-

therefore, most robust for N1 during the presentation of negative odors (Pause et al., 2008). Among alcoholics, electrophysiological data showed delayed olfactory N1-P2 latencies and reduced P2 amplitude (Maurage et al., 2011). In healthy adults, studies found that the OERP vary with age and gender. Compared to older adults or men, younger adults or women have generally greater amplitude and shorter latency (Covington et al., 1999; Morgan et al., 2010; Murphy et al., 2000; Olofsson and Nordin, 2004; Stuck et al., 2006).

In this paper, we would like to investigate how the duration time of odor mixture modulates central nervous stimulus processing. There are two questions we want to explore in this paper. First, will the different duration time of odor presentation affect the speed and strength of the odor identification? Second, will the different duration time of odor presentation affect the evaluation of the odor concentration?

2. Material and methods

2.1. Subjects

Twenty-one right-handed males (mean age, 19.2; SD, 0.62 years; age range, 19-21) were recruited from an academic environment. All subjects were free from neurological and psychiatric disorders, no smokers, had normal vision or corrected visual acuity, no color blindness and color weakness, and gave informed consent to participation in the study. The study was approved by the local ethics committee.

2.2. Olfactory experience tester

The olfactory experience tester used in this experiment is the OET-1.0 model, which is developed by a team come from Chongqing university. This device can simultaneously hold 10 odor sources in maximum. Odors used in the experiment can be controlled through external computer control or direct use of LCD screen. Pipes and mask are connected at the back of the equipment, which reduce the impact of external environment and increase the accuracy of odor perception.

Set up the audio for different frequency, timbre, pitch and volume. By taking the Fourier transform of the time domain waveform of audio signal, receive the frequency spectrum of the frequency domain. Because different audio signals have different frequencies, they can control the operation of different air pump motor drivers, and realize the function of different sound frequencies control the operation of air pump motors. At the same time, the duration of the audio signal is equal to the smell release time during the experiment. After the audio signal processing, the release of odor can be controlled precisely. This method has simple structure and control is convenient. After programming in E-prime, computer sends an audio signal to the olfactory experience tester. Through signal analysis and processing, control the operation of air pump on the basis of received frequency, release corresponding odor and perceived by the participant. Three channels used in this experiment are 1000 Hz, 2000 Hz and 3000 Hz respectively.

2.3. Study design (stimuli and procedure)

The experimental program is divided into 10 blocks, each block present 60 times stimulation, three kinds of stimulus (standard: apple sweet; target: sulfur soap; distractor: cigarette; each stimulus has two duration time: 300 ms and 500 ms) appeared in each block randomly. In addition, the introduction of odor is mainly caused by three different frequencies of sound triggering the olfactory generator. The experiment was designed with three odors (apple (70%), sulfur soap (20%), cigarette (10%)) * 2 odor duration (300 ms/500 ms). The frequency of three different sounds corresponding to the three different smells is 1000, 2000 and 3000 Hz in advance. The experimental program used is E-prime 2.0 software.

In order to let the participants familiarize with experimental procedures, we first let the participant practice 12 trials before the real experiment started. No extraneous stimulus was present during the trail procedure. Half percent of cigarette, sulfur soap and apple odors account for each duration (apple odor 300 ms – 3 times, apple odor 500 ms – 3 times), stimulus were presented randomly.

Subjects were given acoustic earplugs to wear before the experiment, firstly a ‘+’ gaze fixation point appeared on the screen and existed for 3 s. This prompted the subject to start the test. Then an image of nose presented on the screen, which indicated the emergence of smell, the smell was delivered to the participant through the smell generator. Because prior determined odor needs about 100 ms to be transmitted to the nose, smell the incoming time is respectively 400 and 600 ms, immediately after the participants in the smell key, if sulfur soap flavor press the blank space key, if
other taste does not button. The image of nose continued to show on the screen for 1500 ms, a stage of smell perception. It then automatically jumps to the subjective report screen, and the subject needs to assess the smell of the scent (7 points), the blank space button disappears, and starts the next trial.

2.4. EEG recording and analysis

Electroencephalography (EEG) activity was recorded using a 64 channel BrainAmp amplifier (Brain Products, Munich, Germany) with a 64 electrode Braincap. The electrodes were placed according to the 10-20 System and referenced during recording to an additional reference electrode placed GND (Ground) site, with a forehead ground and impedance of less than 10 kΩ. Additional electrodes were placed on the outer canthi of the two eyes and on the infraorbital ridges of the right eye to record the horizontal and vertical EOG (electrooculography). No filter was used during recording. The EEG were digitized with a sampling rate of 500 Hz. All experimental runs were recorded with eyes open, and rest periods were provided between experimental runs.

EEG data were analyzed by ERPLAB, which is a popular open source tool kit for handling and analyzing event related potential (ERP) data in MATLAB environment. The EEG data was filtered at 0.01–30 Hz (slope 12 dB/ octave) and recalculated using linked mastoids as reference (TP9 + TP10)/2. The analyzed time epoch for each event was 2000 ms (500 ms pre-stimulus and 1500 ms post-stimulus). To avoid body movement and other artifacts, all epochs exceeding 100 μV in any channel were excluded from further analysis. For each epoch, a baseline correction for the data 500 ms prior the stimulus was performed.

For the calculation of the ERPs, the waveforms were averaged off-line. The ERPs were computed separately for the different stimulus and time durations. Based on the evaluation of voltage scalp distributions and existing knowledge about brain structures involved in olfactory processing, we chose two regions for the further analysis and statistics. Firstly, a frontotemporal area (containing electrodes AF7, F5, FC5 and FT7 and their right-hemisphere equivalents) known to be activated in various cognitive odour tasks such as odour identification and odour discrimination was chosen to assess a possible modality-dependent cortical activation representing the perceived olfactory stimulus. Secondly, we choose the three midline electrodes (Fz, Cz, Pz) to analyze further. According to previous studies, N1, P2 is sensory components and N2, P3 is cognitive components for the processing of odor stimuli. In this study, the ERP amplitude and latency data were assessed with a three-factor repeated measure (3 stimulus types [apple, sulfur sofa, cigarette] * duration time (300 ms, 500 ms) * 3 electrodes (Fz, Cz, Pz)) analysis of variance (ANOVA). In addition, in order to compare lateralization aspects of olfactory processing reported in previous studies (Royer and Pailly, 2004; Savic et al., 2002), we averaged the four electrodes in frontotemporal area and named it LFT in left-hemisphere and RFT in right-hemisphere. The N2 component was defined as the largest negative deflection occurring within the time window between 450 and 730 ms. The peak amplitude was measured relative to the pre-stimulus baseline. The peak latency was defined as the time from stimulus onset to the peak of the N2 component. The P3 component was defined as the largest positive deflection occurring within the time window between 730 and 1050 ms. The peak amplitude was measured relative to the pre-stimulus baseline. The peak latency was defined as the time from stimulus onset to the peak of the P3 component. As for N1 and P2 components, the amplitude time window is 110-180 ms and 180-260 ms. The corrected P values are reported in results.

3. Results

3.1. Behavioral data

The analysis of the variance of the two factors of stimulation and duration time was performed on the stimulus concentration, the results show that the duration time has a significant main effect \(F(1,20)= 23.31, p < 0.001, \text{nt}^2 = 0.45\), odor presented under the condition of 300 ms, in which score of concentration (M = 3.42) was significantly lower than the odor concentration presented under the condition of 500 ms (M = 3.78, p < 0.001).

3.2. ERP results

For each component (N1, P2, N2, P3), \(3 \times 3 \times 2\) ANOVAs were computed separately for latencies, peak and amplitudes, with electrode (Fz, Cz, Pz) and stimulus type (standard, target, distractor) and duration time (300 ms, 500 ms). The waveform diagram of each stimulus and duration time in the midline sites and the topographic map of target’s P3 amplitude are presented in Fig. 2.

3.3. N1

- Latency. There was neither significant main effect nor significant interaction.
- Peak. A significant main effect was found for the electrodes \(F(2,40) = 17.427, p < 0.01; \text{nt}^2 = 0.466\): the peak is most negative at Fz then Cz , the last Pz sites. The main effect for duration time is edge significantly \(F(1,20) = 4.143; p = 0.055\): the peak in 500 ms duration time is more negative than the 300 ms condition.
- Amplitude. There was a significant main effect for the electrodes \(F(2,40) = 17.427; p < 0.01; \text{nt}^2 = 0.466\): the amplitude is most negative at Fz then Cz , the last Pz sites. There was neither significant main effect nor significant interaction for stimulus and the duration time condition.

3.4. P2

- Latency. There was neither significant main effect nor significant interaction.
- Peak. There was a significant main effect for stimulus \(F(2,40) = 6.557; p < 0.01; \text{nt}^2 = 0.247\) and electrode \(F(2,40) = 4.387; p < 0.05; \text{nt}^2 = 0.18\): distractor had a higher peak than the standard odor; the peak at Cz sites is more positive than the Fz sites.
- Amplitude. A significant main effect was found for the Electrode \(F(2,40) = 9.065; p < 0.01: \text{P}2\) amplitude was more positive at Cz and Pz than Fz. There was no significant main effect or significant interaction for stimulus and the duration time condition.

3.5. N2

- Latency. A significant main effect was found for duration time \(F(1,20) = 11.815, p < 0.01, \text{nt}^2 = 0.371\): N2 latencies were longer as the duration time of stimulus increased. Moreover, a significant duration time × electrode interaction \(F(2,40) = 7.99; p < 0.01\) indicated that only at the Fz and Cz sites, the 500 ms duration time condition presented longer N2 latencies than the 300
ms condition, while the two duration time condition had no differ at Pz sites.

- Peak. A significant main effect was found for duration time $[F(1,20) = 4.773, p < 0.05, \eta^2 = 0.193]$ and electrodes $[F(2,40) = 27.202; p < 0.01; \eta^2 = 0.576]$: N2 peak in 500 ms condition was more negative than the 300 ms condition and the peak was getting smaller from Fz to Pz sites.

- Amplitude. A significant main effect was found for duration time $[F(1,20) = 5.653, p < 0.05, \eta^2 = 0.22]$ and electrodes $[F(2,40) = 22.677; p < 0.01; \eta^2 = 0.531]$: N2 amplitude in 500 ms condition was more negative than the 300 ms condition and the amplitude was getting smaller from Fz to Pz sites.
3.6. P3

- Latency. There was a significant main effect of the duration time \(F_{(1,20)} = 5.006, p < 0.05, n^2 = 0.2\), the 300 ms condition (P3 latency : 881.10 ms) had a significantly shorter latency compared to the 500 ms presentation time (P3 latency : 904.36 ms). Fig. 1 presents the grand average ERP waves for three odors from three selected electrodes (Fz, Cz, and Pz) and the P3 amplitude scalp maps for the target stimuli in different duration time.

- Peak. A significant main effect was found for stimulus \(F_{(2,40)} = 15.16; p < 0.01, n^2 = 0.431\] and electrodes \(F_{(2,40)} = 9.696; p < 0.01; n^2 = 0.327\]: the P3 peak is getting bigger from the Fz to Pz. The distractor has a higher peak than standard odor at Fz, while the peak of distractor was bigger than standard and target stimuli at Cz. At Pz sites, target stimuli were higher than standard stimuli, but lower than the distractor odor. As for the different duration time, there is no significant differ at the P3 peak.

- Amplitude. Repeated measurement ANOVA reveals a significant main effect of the stimulus \(F_{(2,40)} = 3.396, p < 0.05, n^2 = 0.145\] and electrode \(F_{(2,40)} = 16.624, p < 0.01, n^2 = 0.454\]. By paired comparisons, we found that the P3 amplitude significantly increased from Fz to Pz, and the cigarette has larger P3 amplitude than the apple odor in Cz and Pz electrodes. P3 amplitude has no significant difference between the two different duration times (300 ms, 500 ms) about the same odor stimuli.

3.7. Lateralization

For N2 components, 3 \(\times\) 2 \(\times\) 2 ANOVAs were computed separately for latency and amplitude, with stimulus type (standard, target, distractor), duration time (300 ms, 500 ms) and lateralization (LFT, RFT). We found a significant main effect for duration time \(F_{(1,20)} = 4.83, p < 0.05, n^2 = 0.195\] and lateralization \(F_{(1,20)} = 8.153, (1.20) < 0.05, n^2 = 0.29\]. The waveform diagram and topographic map for N2 component of target stimuli at different duration time is presented in Fig. 2. Moreover, the duration time \(\times\) lateralization had a significant interaction \(F_{(1,20)} = 4.361, (1.20) < 0.05, n^2 = 0.179\], which revealed that only at left frontal-temporal the 500 ms condition had a more negative amplitude than the 300 ms condition. As for the N2 latency, there was no significant main effect nor significant interaction. The waveform diagram of target stimulus in different time duration at LFT (left frontal-temporal) and RFT (right frontal-temporal), besides, the topographic map for N2 component are presented in Fig. 2.

4. Discussion

The major finding of this research is that the duration time of odor mixture has a significant effect on the central nervous olfactory processing. We have explored the ERPs components of the odor mixture using a standard oddball paradigm and found that odor mixture induced the similar components compared to other modality stimulus. There were two type components for the visual and auditory stimuli in the oddball paradigm, one is exogenous components which related to the properties of the stimuli, the other is endogenous components which reflected the cognitive processing for the stimuli (Barry et al., 2003; Campanella et al., 2002).

We found that the exogenous components N1 and P2 just like the visual and auditory stimuli induced, while N1 and P2 components here are distinctly different compared to the earlier chemosensory or olfactory study using single molecule olfactory or trigeminal stimulation (Kayser et al., 2010; Maurage et al., 2011; Rombaux et al., 2006). The properties of odor mixture in the two duration time condition have no difference. As a result, the duration time has no effect on the N1 and P2 component, which further confirmed that the N1 and P2 here are completely the exogenous components rely on the properties of stimuli (Hirata and Lehmann, 1990). However, the earlier chemosensory and olfactory study using single molecule stimuli has named a negative deflection wave occurs between 320 and 450 ms after stimulus onset as N1, a positive deflection wave occurs between 530 and 800 ms after stimulus onset as P2. The latency of N2 and P3 components here is similar to the N1 and P2 occur time in earlier chemosensory or olfactory studies using single molecule odor stimulus. Interestingly, some studies have explained that the olfactory and chemosensory N1 could be regarded as olfactory N2, analogous to a visual or auditory N2, while the P2 would be equivalent to a P3 complex typically observed during many ERP paradigms, including an oddball task. All in all, we found the similarity of ERP components using odor mixture compared to the study using visual or auditory stimuli within oddball task. Moreover, the N2 and P3 components here could be regarded as the N1 and P2 components in the earlier chemosensory or olfactory studies.

When the odor mixture was released, a picture of the nose was presented to remind the participants. Simultaneously, the onset of odor mixture accompanied by a weak vocal stimulus, we have used earplugs to avoid the effects of sound irritation. As for the N1 and P2 in this study, they are maybe the reaction to the picture or the properties of odor mixture. While the N2 and P3 are related to the nervous processing of odor stimuli.

The duration time had a significant influence on the latency and amplitude of the N2 component in this study. The latency of N2 and P3 becomes longer as the duration time of odor stimuli extended. ERP latency reflects the processing speed while ERP amplitude indexes the processing intensity, i.e. the neuronal population implied in this processing stage (Rugg and Coles, 1996). There is no doubt that the extension of the stimulus duration time has led to a delay in the speed of cognitive processing. While considering the longer duration time of odor stimulus has larger amplitude than the shorter condition, this effect is significant in the mid-frontal, mid-central cortex areas, as well as the left frontal-temporal areas. The N1 component in the earlier chemosensory and olfactory study is associated with the odor perception, identification and discrimination, moreover, the amplitude becomes larger as the concentration of odor stimuli increased. In this study, the longer duration time has larger amplitude than the shorter condition, maybe the concentration has become higher as the presentation time longer. As to the lateralization of odor mixture processes, the left hemisphere presents more activation than the right frontal-temporal, a review has revealed that there is a dissociation of olfactory process, with involvement of the right hemisphere in memory processes and the left hemisphere in emotional processes (Anderson et al., 2003; Royet and Pailly, 2004). The duration time has a significant effect on the N2 amplitude in the left hemisphere rather than the right side, which suggests that the longer presentation of odor mixture may elicit stronger emotions compared to the shorter duration time. As for the emotional valence, we need
to further explore in the future study.

The P3 amplitude increased from the frontal to parietal recording sites, which is consistent with the olfactory study using single-stimulus oddball paradigms (Morgan et al., 2010). P3 latency is a sensitive temporal measure of neural activity underlying the processes of attention allocation and working memory update (Polich et al., 2010). The longer duration time of odor stimuli significantly extended the latency of P3 component, which reflected that the longer presentation of odor stimuli delayed the cognitive processing in neural activity. As for the P3 amplitude, there was no effect of the duration time in this study, this effect may be explained by the function of P3 components. From the topography and statistics of P3 components, the largest amplitude is located in the mid-parietal area, which is compatible with the notion of a close association of olfactory P2 with a classical P3b potential (Lorig, 2000; Olofsson et al., 2008). The N2 amplitude was elevated by the longer duration time of stimuli, the P3 amplitude was not influenced by the different duration time, which suggests that the evaluation of working memory related to the neural processing of odor concentration was independent on the duration time of stimuli.

5. Conclusions

We found that longer duration time delayed the neural cognitive process, while strengthening the activity of mid-frontal and left frontal-temporal cortex regions. However, there was no effect
on the amplitude of the late positive wave, which indicates that the duration time had no influence on the evaluation of odor mixtures and working memory related to the neural processing of odor concentration.

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Conflict of Interest
The authors claim no conflict of interest.

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