Exploring human brain activities involved in the hedonic-evaluating process of odors

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Abstract
There are several studies showing the hedonics of odor affects human brain responses. In preceding studies, however, the hedonics of odor was decided by experimenters' settings, not by participants' own ratings. This might be one reason of disagreement of activated brain areas among preceding studies. Thus, in this study, the odor stimulations were grouped into one of three conditions, pleasant, neutral and unpleasant conditions, based on participants' own ratings, and then brain responses revealed by fMRI were analyzed and compared based on these conditions. Activation was clearly detected in the insula and the cingulate cortex during odor stimulations that participants evaluated as unpleasant, and in the cingulate cortex during odor stimulation that the participants evaluated as pleasant. It is suggested that the inconsistency of activated brain areas in previous studies may have been caused by individual differences in the evaluation of odor hedonics.

Introduction
Many studies have reported human responses resulting from the hedonic aspects of olfactory processing. For example, the perception of unpleasant odors elicits stronger autonomic responses, such as changes in skin conductance, skin temperature, blood pressure, and pitch of voices. These results show that the hedonic aspects of odor can affect the human emotional system. Furthermore, it has been demonstrated in other species that odor processing directly activates primitive and emotional regions of the cerebral cortex. Thus, studying the processing of the hedonic aspects of odor may help us understand the mechanisms underlying human emotional response.

There are also many researches studying human brain response involved in processing hedonic aspects of odor. Using positron emission tomography (PET), it was found that perception of odors which evoke emotional response in human elicits strong regional blood flow (rCBF) in the orbitofrontal cortex, the amygdala, and the cerebellum. The difference between responses to pleasant odor and unpleasant odor, however, was not considered in the preceding studies.

Studies with functional magnetic resonance imaging (fMRI) revealed that several brain areas showed signal changes related to the hedonics of the odor. For example, Brodmann's Area (BA) 8 in both hemispheres was suggested to be involved in the processing of pleasant odors, while BA 6 in the left hemisphere and BA 32 in the right hemisphere were suggested to be involved in processing unpleasant odors. Kettenmann and her colleagues measured activities of the human brain with electroencephalography (EEG) and magnetoencephalography (MEG) during odor stimulation and found that the second component of olfactory event-related EEG responses was larger during stimulation of pleasant odor than that of unpleasant odor. During MEG measurements, consistent equivalent current dipoles were located in the anterior-central
part of the left insula. Another EEG study found stronger activation of the left frontal hemisphere during the presentation of a pleasant odor, than the activation measured during presentation of an unpleasant odor or a neutral odor. Indeed, these studies divided odor stimuli into pleasant odor and unpleasant odor, and compared the results of pleasant odor with unpleasant odor, but they did not regard participants' evaluation of hedonics as significant.

In terms of the mechanisms underlying emotion, some questions remained unanswered by the preceding studies. First, possibly owing to differences in the detection methods, few common areas, which were activated by pleasant odor and/or unpleasant odor, were found across these studies. Because MEG has a limited ability to detect the signals from structures deep in the brain, such as the amygdala, the caudate nucleus, and the putamen, contributions of these areas could not be clarified with MEG measurements. We, therefore, used magnetic resonance (MR) imaging as a tool to identify brain regions involved in processing the hedonic aspects of odors. fMRI also has advantages over PET by its less invasiveness. Because fMRI uses the difference between the magnetic resonance signals of oxy-hemoglobin and deoxy-hemoglobin to locate brain areas that are active, it is less invasive than PET, which uses positron emission. A second problem is that although the insula of the left hemisphere was found by both Fulbright et al. and Kettenmann et al. to be involved in processing of odor pleasantness, these studies used only two kinds of pleasant odor (clementine and vanillin, respectively). Thus, it remains to be seen whether the relative pleasantness or relative intensity of an odor leads to activation of the insula. Thus, we used a new set of odor stimuli and examined whether or not the insula was also activated by these odors.

The third problem is that, in the preceding studies, it was the experimenters, not the participants, who decided whether the odors were pleasant or unpleasant. Because there was no data about participants' own evaluation of the stimuli, we do not know if the participants really evaluated a pleasant odor as pleasant and an unpleasant odor as unpleasant during odor stimulation. Thus, the activated areas found in these studies might reflect the processing of the quality of the odors, not the hedonics of the odors. Recently, functional difference within orbitofrontal cortex in processing pleasantness-unpleasantness of odor with several pleasant and unpleasant odors were investigated. However the study did not examine signal changes in other regions of the brain. Thus, in this study, signal changes in all brain regions are analyzed during odor stimulations with taking each individual's hedonic evaluations into account.

I. Methods

Participants

Thirteen university students (9 males and 4 females) participated in this study. Each participant was evaluated for their overall physical condition and tested for the ability to smell the odors used in this study.

Stimuli

Three odors were used: phenyl ethyl alcohol (PEA), which has a rose-like odor, citral (CIT), which has a citrus-like odor, and triethylamine (TEA), which has the odor of rotten fish. The odors were presented by bubbling an undiluted odor solution and diluting the resulting odor gas. The flow rates of the odor stimuli were 4 L/min. The three odor stimuli were randomly presented with the olfactometer. The olfactometer could deliver the odor in an intermittent pattern. In an unpublished preceding study, the participants were asked to evaluate the subjective intensity for the odors presented by this olfactometer. When the odors were presented in an intermittent pattern (40 seconds with odor, 40 seconds without odor), the participants evaluated the intensity in an intermittent pattern. An example of this preceding experiment was shown in Figure 1.

Each participant underwent four sessions of odor presentation for each of the three odors. Thus, there were twelve 80-second sessions for each participant in this experiment. During each session, the odors were presented for 40 seconds followed by the presentation of odorless
air for 40 seconds.

**Procedure**

Participants were allowed to practice smelling the odors and familiarized with the odor evaluation scale. Information about the experiment, including the aim of the study, an explanation about the MR scanner, instructions for experiment, and the participants' rights, were given orally and on paper. All the participants then gave their written consent to partake in the study. This study was approved by the institutional review board at the National Institute of Advanced Industrial Science and Technology.

During odor presentation, participants were asked to smell each odor and evaluate its pleasantness and intensity. The scale was a quasi-analogue scale: from −3 (completely unpalatable) to +3 (completely pleasant) for hedonic evaluation, and from 0 (no odor) to 5 (very strong) for intensity estimation. 20-minute intervals after each odor stimulation allowed olfaction recovery and the rating of odor pleasantness.

Based on each participant’s personal evaluation of the odor stimuli, the presentations of odors were divided into one of the following conditions: a pleasant condition (evaluated ratings ≥+1), a neutral condition (ratings > −1 and < +1), and an unpleasant condition (ratings ≤ −1).

**MR recording**

Echo-planar images (EPI) were taken with a 1.0 Tesla fMRI scanner (Siemens Magnetom Impact). Twenty horizontal EPI were acquired every four seconds with a slice thickness of 4 mm. Thus, there are 20 whole brain scans from each session, and 80 scans for each odor. The matrix size was 64×64. After acquiring EPI during odor stimulation, one horizontal T2* weighted image was acquired as an anatomical reference.

**Image analysis**

The images were analyzed with statistical parametric mapping (SPM2). Image processing included realign-
ment, spatial normalization, and smoothing of the images using a three-dimensional Gaussian filter (FWHM, 8 mm). Images were discarded if a change in the position of the in-plane center of the mass exceeded 1 mm. A statistical parametric map was generated by fitting a box-car function to each combined dataset. Significant changes of voxel intensity between each odor and odorless air were calculated. The threshold for the resulting z-maps was \( P < 0.05 \) (uncorrected) for each odor and for each participant. Then, second level analyses, in which the contrast images for each odor and for each condition were produced by summarizing all participants' contrast images, were performed. Some previous studies have suggested consistently that stimulation with pleasant or unpleasant odors increases brain activity in the following emotion-related brain regions: the insula, the amygdala, the cingulate cortex and the prefrontal cortex including the orbitofrontal cortex. Thus, in these second level analyses, region of interest (ROI) were determined in these olfactory-related areas. The threshold for the resulting z-maps was \( P < 0.05 \) (corrected) in each case. The coordinate parameters are based on the guideline from the Montreal Neurological Institute (MNI).

II. Results

Because images were discarded if a change in the position of the in-plane center of the mass exceeded 1 mm, five scans were not used in this study. Thus, scans obtained during 34 stimulations were analyzed.

Odor Evaluations by Participants

Ten participants evaluated TEA as unpleasant, one participant evaluated TEA as neutral, and no one evaluated TEA as pleasant. For CIT, one participant evaluated the odor as unpleasant, five participants evaluated the odor as neutral, and five participants evaluated the odor as pleasant. Two participants evaluated PEA as unpleasant, two participants evaluated PEA as neutral, and eight participants evaluated PEA as pleasant. Thus, we obtained useful data sets from thirteen unpleasant condition trials and thirteen pleasant condition trials.

The averaged (±SD) hedonics scores for each condition were \(-1.8±0.8\) (unpleasant), \(0.1±0.2\) (neutral) and \(1.3±0.5\) (pleasant). The statistical analysis (ANOVA) confirmed this segregation (\( F(2,31)=93.3, p<.001 \)). The hedonic ratings for odors classified as unpleasant were significantly lower than those rated as pleasant or neutral (\( p<.001 \)). Additionally, the hedonic ratings for pleasant odors were significantly higher than neutral odors (\( p<.001 \)).

The averaged (±SD) intensity scores for each condition were \(3.6±0.2\) (unpleasant), \(3.4±0.3\) (neutral) and \(2.8±0.2\) (pleasant). A significant difference was found in the intensity ratings assigned to the different odor conditions (\( F(2,31)=4.11, p<.05 \)). The intensity ratings for unpleasant odors were significantly higher than those of pleasant odors (\( p<.05 \)).

Activated Brain Areas Revealed by fMRI

Several brain regions showed significant changes in their voxel intensity during odor stimulation. Examples of significant signal changes after performing simple analyses in single participant are illustrated in Figure 2. The signal showed a correlate change to the odor stimulations.

Then the signal changes were analyzed by the second level analysis. In this analysis, the contrasts from all participants for each odorant (PEA, CIT and TEA) or each hedonic value (pleasant, neutral and unpleasant) were used.

The results about analysis based on odorant were shown in Table 1. Table 1a indicates the activated brain regions to PEA stimulation (\( F>88.60, p<.05, \) voxel > 8). The signal changes responding to PEA stimulation were found in left middle frontal gyrus. Table 1b indicates the activated brain regions to CIT stimulation (\( F>88.94, p<.05, \) voxel > 8). The signal changes responding to CIT stimulation were found in cingulate cortex (Broadmann Area 32 and 33). Table 1c indicates the activated brain regions to TEA stimulation (\( F>76.83, p<.05, \) voxel > 8). The signal changes responding to TEA stimulation were found in insula and cingulate cortex.

The results about analysis based on participants' own
hedonic ratings were shown in Table 2. Table 2a indicates the activated brain regions to unpleasantness by odor stimulation \((F > 61.78, p < .05, \text{ voxel } > 8)\). The signal changes responding to unpleasantness were found in right insula, right amygdala and cingulate cortex. Table 2b indicates the activated brain regions to pleasantness by odor stimulation \((F > 76.51, p < .05, \text{ voxel } > 8)\). The signal changes related to pleasantness were found in cingulate cortex. The signal changes responded to neutral (neither pleasant nor unpleasant) were not found in ROI.
Table 2. Brain areas that showed signal changes during odor stimulation when the data were analyzed based on participants' own pleasantness ratings. The upper panel (a) shows results for unpleasantness, and the lower panel (b) shows results for pleasantness. There are no activated voxels in neutral condition. The spatial coordinate parameters (x, y, z) are based on MNI.

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<th>Voxels</th>
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<th>x</th>
<th>y</th>
<th>z</th>
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(F > 183.56, p < .05, voxel > 8). Some of the activated areas are shown in Figure 3.

The number of activated voxels responded to unpleasantness were more than those to pleasantness (177 voxels for unpleasantness vs 35 voxels for pleasantness).

![A: Unpleasantness](image1.png)

![B: Pleasantness](image2.png)

Figure 3. Brain signal changes around the insula (Ins), amygdala (Amy), anterior cingulate (AC) and cingulate cortex (CC) during odor stimulation. The white circle indicates significant signal changes. Left panel (A) showed signal changes of unpleasant condition, and right panel (B) showed pleasant condition.
III. Discussion

In this study, we have confirmed some previously reported results by examining brain activity during the presentation of odors that the participants themselves hedonically evaluated. The number of the activated voxels in unpleasantness condition was greater than those of pleasantness condition. This result suggests that more voxels are activated in response to odor stimulation, the participant evaluate the intensity for the odor stronger.

The activated brain area showed larger differences when the second analysis was done based on the participants’ own pleasantness ratings than based on the odorant. A minute discussion about each activated brain area was as following.

**Insula**

We observed signal changes around the insula in both of emotional conditions (pleasant and unpleasant). However, the signal changes were only significant during presentations of the odors that the participants evaluated as unpleasant. This result was consistent with previous studies that reported the involvement of the insula in the processing of unpleasant emotions elicited by visual stimuli or unpleasant memories.

In a preceding study, it was found that the size of dipole measured with MEG in insula was different during stimulation by pleasant odors and unpleasant odors. Also in other study, signal changes with fMRI in insula from both hemispheres were detected after clementine stimulation, while isovaleric-acid stimulation only produced changes in the right insula. These results suggested that the left insula is involved in processing pleasant odor perception while the right insula has a more general role processing odor stimulation. In consistent with these results, we detected significant signal changes in the right insula during presentations of unpleasant odors. However, we could not detect significant signal changes in the left insula in pleasant condition.

**Amygdala**

Although activity changes were observed in the amygdala when the participants evaluated the odor stimuli as unpleasant, there were no activated voxels in the amygdala during neutral or pleasant odor stimulation. This result confirmed the results of preceding studies. In the first study investigating human brain response to odor stimulation with PET, signal changes were found in the amygdala of both hemispheres after highly aversive odor stimulation. Moreover, some preceding studies have suggested that the amygdala is involved in the processing of unpleasant emotions.

**Frontal (or Prefrontal) Cortex**

Lateral prefrontal cortex of left hemispheres showed signal changes only after rose–like odor stimulation (PEA). O'Doherty and his colleagues reported that olfactory stimulation with vanilla and banana odors evoked signal changes in the lateral prefrontal cortex of both hemispheres. Thus, it is suggested that the lateral prefrontal cortex shows response to the odor stimulation. On the other hand, some experiments showed stimulation of pleasant odors elicited signal changes in middle orbitofrontal cortex. It is still needed to clarify functional differences within orbitofrontal regions of prefrontal cortex. The MR scanners and the methods of scanning (single–shot of EPI) used by many researchers were not suitable for recording signals around the orbitofrontal cortex because of signal distortion. The inconsistency of the results could be caused by this problem. Future studies will attempt to use some technical improvements, such as z–shimming acquisition to record from the orbitofrontal cortex.

**Cingulate Cortex**

In this study, cingulate cortex showed significant signal changes in many conditions, such as pleasant, unpleasant, CIT and TEA. This result was consistent with those of many preceding studies. Thus, the cingulate cortex is suggested to be involved in processing olfactory information, but its role in olfactory processing remains unclear. A study investigating the effect of age on odor–induced signal changes by fMRI showed the number of activated...
voxels in the cingulate cortex of the younger group was greater than those of the older group. The further researches studying the relationships between activations in the cingulate cortex and cognitive aspect of olfaction, such as odor memory, odor images and odor identifications, are needed.

Individual difference of odor perception

The inconsistency of activated brain areas in previous studies may have been caused partially by individual differences including hedonic strength in the evaluation of odor hedonics. Several studies that considered individual differences in odor hedonics have been reported. Moreover, differences in odor hedonics have been suggested to cause differences odor recognition, odor adaptation, odor intensity and hedonic ratings, and odor-induced signal changes in the insula, the amygdala and the cingulate cortex. Thus, differences in odor perception by individuals must be considered during studies of the brain activity induced odor stimuli using the same analytical method as this study.

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