Ph. D Thesis

박사 학위논문

# Studies of the odor information processing in the human brain based on EEG activity

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2021

# Studies of the odor information processing in the human brain based on EEG activity

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by

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A thesis submitted to the faculty of DGIST in partial fulfillment of the requirements for the degree of Master of Science in the Department of Energy Science & Engineering. The study was conducted in accordance with Code of Research Ethics[1]

11. 23. 2020

Approved by

Professor Cheil Moon (signature)
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[1] Declaration of Ethical Conduct in Research: I, as a graduate student of DGIST, hereby declare that I have not committed any acts that may damage the credibility of my research. These include, but are not limited to: falsification, thesis written by some-one else, distortion of research findings or plagiarism. I affirm that my thesis contains honest conclusions based on my own careful research under the guidance of my thesis advisor.

# Studies of the odor information processing in the human brain based on EEG activity

Jisub Bae

Accepted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy.

11. 23. 2020

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Ph.D/BS
201325007

배 지 섭. Jisub Bae. Studies of the odor quality processing in the brain based on temporal view. Department of Brain & Cognitive Sciences. 2021. 123p. Advisors Prof. Cheil Moon, Prof. Co-Advisors Ji-Woong Choi.

**Abstract**

Every day we discern and recognize objects. But how objectively can we perceive and distinguish them? And how can we objectively describe these objects to others? This thesis started from these questions and tried to find the answers by analyzing the sense of smell, which is known to be more subjective than other senses. Therefore, I focused on finding a general mechanism of differentiating odors by checking cognitive features using brain signals. I especially focused on the changing temporal pattern of brain signals. Our nerves encode information using both spatial and temporal methods, and our brain performs many different functions depending on which part is activated and when. In humans, temporal brain signals have been relatively less studied than spatial brain signals; thus, this study used electroencephalography (EEG) because of its good temporal resolution.

The first study examined whether the olfactory signal can be directly measured by means of the EEG signal. Because olfactory signals start to be processed from the deep brain areas, the EEG signal can be weakened. Moreover, some in vitro studies suggested that olfactory processing in the brain starts from over 200ms, although recent MEG or behavioral studies suggest that olfactory processing starts before 200 ms. Thus, it is necessary to verify whether the olfactory signal can be directly measured before 200 ms by EEG signal. From this study, I found that the olfactory-specific signal was measured before 200 ms and was observed to be changed by changes in the olfactory stimulus; it was also verified that the olfactory signal before 200 ms can be directly measured with EEG.

In the second study, based on first study, the odor categorization mechanism was addressed and confirmed in terms of time. Two similar odors and one completely different odor were selected and the corresponding EEG signals were measured. I found that two similar odors showed similar pattern at time range of 50–100 ms, 150-200ms and 350-400ms in theta. The gamma wave also showed similar pattern at 100–150ms and 350–400 ms. Moreover, these results were related with olfactory related brain areas. These results revealed that odor discrimination processed by each olfactory related brain areas, especially at a specific time range.

In the third study, I focused on characterizing odor in the behavioral and survey level. Although I verified that differentiating process of odor can be represented by EEG, the object of my thesis is to add a greater understanding of odor information processing. Ever-increasing physiological and behavioral studies suggested several features for characterizing odor quality (including study 1 and 2), but there are no precise methods for measuring the multidimensional axis of odor quality. Moreover, this issue has other difficult problems that odor quality can be altered by individual experience. Therefore, to clarify the preceding question, I tried to quantify and determine the odor responses by alteration using verbal cues. I found that the odor descriptors with a high score (top 25%) was not changed significantly by verbal cues, and using this finding, I suggest that odor quality can be characterized.

These studies suggest that theta and gamma are important frequency bands in the early stages of odor categorization. In particular, between 50 and 100 ms is the active time zone in which the primary olfactory cortex first starts to be activated during olfactory signal processing. This means that odor categorization can be clearly performed before interacting with cognitive functions such as memory during the olfactory process, and that it can be an objective odor categorization index through the activation pattern at 50–100 ms. Therefore, I suggest that people perceive odor objectively at least in the 50–100 ms period after odor recognition. Moreover, although less evidence, I found that these central olfactory processing features may be related to final behavior output.

Keyword: Odor object quality, characterizing odor quality, olfactory processing, temporal pattern coding, human brain

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# I. Introduction

## “Can you describe an objectively?”

When you look at this photo, what do you see? Do you see it objectively? If you are convinced about what you perceived from this photo, can you objectively describe it so that someone can distinguish this photo from other objects?

**Figure 1. An object called 'steak'.** This object can be perceived with all five senses. It is described as brown in color, with a sizzling sound, a soft texture, an umami taste, and has a savory and smoky scent.

Each time we perceive external stimuli, we do so by using our five senses, and these perceptions are modified by numerous cognitive phenomena. Induced cognitive phenomena can be expressed differently according to our experiences or circumstances, and even if the same object is detected at the same time, it can cause different cognitive phenomena for each individual. If this is occurring, how can we convey objective information when explaining and delivering information about a specific object? My question focused on sorting the cognitive index that makes it possible to distinguish objective information from other input among the many cognitive responses that are triggered when we recognize a particular object.

I focused on the olfactory system, a chemical sensory organ as the other physical senses of vision, hearing, and tactile perception are well studied in terms of behavioral and cognitive responses. However, in the case of olfaction, the subjective characteristics are more prominent. In addition, the olfactory organ detects and distinguishes many types of odors, but quantitative differences have not yet been established. This was pointed out in 1914 by Alexander Graham Bell when he said “It is very obvious that we have very many different kinds of smells, all the way from the odor of violets and roses up to asafetida. But until you can measure their likenesses and differences you can have no science of odor”. Thus, the current study was conducted to confirm which cognitive phenomena can be used as an index to distinguish between odors.

As previously mentioned, there are several approaches that may help to answer this question. I have attempted to approach the above question by focusing on the temporal patterns of signaling in the brain. Based on results from rodent studies, it can be concluded that the odors we detect in the nose lose their physicochemical properties when they finally reach the brain. These findings suggest that behavior-based research is essential to determine how we classify and evaluate odors, so I conducted a human study that facilitates direct measurement of odor perception responses. Additionally, by measuring the temporal pattern of brain signals, I confirmed the physiological mechanism of cognitive phenomena. In animal experiments, the gap between temporal and spatial studies was relatively small, but in human studies, this gap is larger, and temporally based studies are more numerous than those location-based studies using fMRI (Figure 2). Neurons use both spatial and temporal activation patterns to encode information, and the brain responds differently as certain 'areas' become 'when' activated. Therefore, the current study examined odor information processing in the human brain through research

based on temporal activation patterns. The following section titled “Aims of the thesis” provides greater detail.

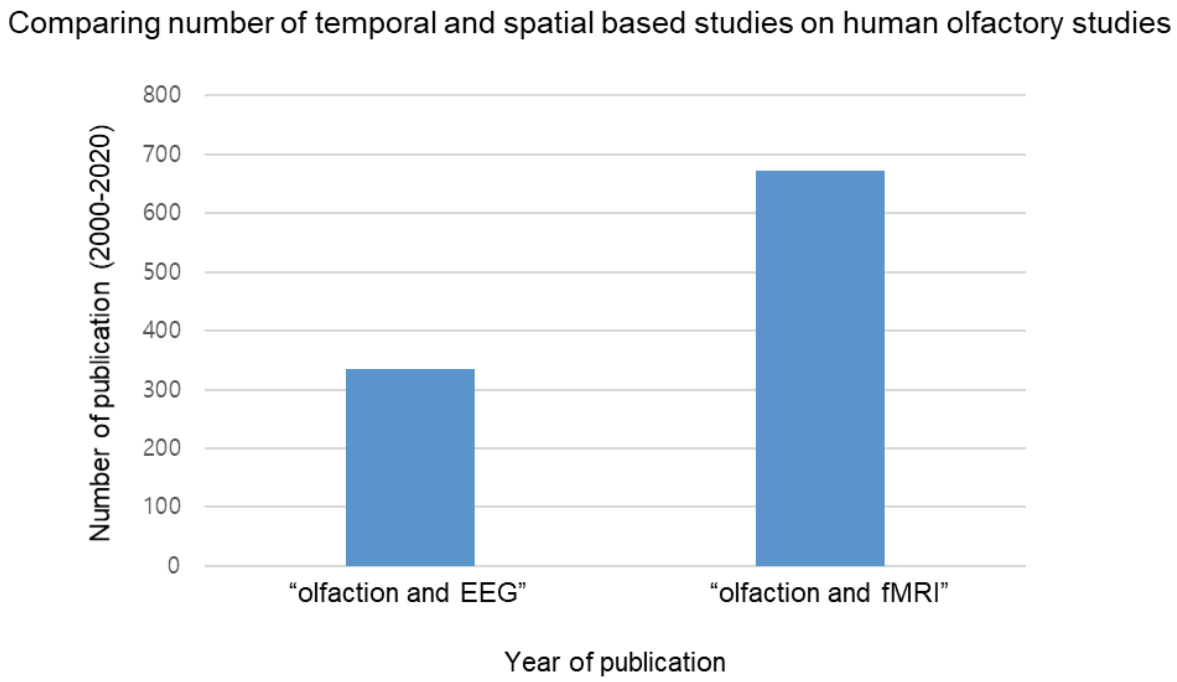



**Figure 2. Comparing the number of temporal and spatial studies on human olfaction.**

# II. Theoretical background

## 1 Our senses

We interact with our environment using our senses. In general, vision, hearing, tactile perception, gustation, and olfaction are recognized as the five basic senses. Indeed, depending on how you define a sense, we have up to 20 different senses, including those that perceive chemosensory irritation in the eyes or blood vessels. However, as highlighted in the introduction, my question focused on perceiving an object objectively using the olfactory sense.

Vision is processed when light (photons) is detected by the eyes. Rods and cones in the retina of the eye detect a narrow band in the electromagnetic spectrum of light. This light signal is transferred to the visual cortex where each receptor on the retina is mapped to specific neurons [13]. Hearing and tactile perception is initiated by the movement of molecules in the world outside the organism which is called 'mechanosensation', where mechanical stimuli are mapped in the sensory cortex [12]. The senses of gustation and olfaction function by detecting chemical changes. Olfaction can detect and discriminate thousands of different chemical signatures. In contrast, gustation functions by recognizing combinations of five different tastes: salty, sweet, sour, bitter, and umami. These tastes are generated by chemosensory receptors which directly correspond to the five tastes, and are mapped to the primary gustatory cortex [23]. However, the olfactory system has a different pathway for processing sensory information. Perceiving an odor begins with the activation of a specific odorant receptor (OR) repertoire rather than activating one individual receptor [35,62,83]. This odor information is also mapping to the olfactory bulb (OB), which processes chemical structure information rather than reflecting our perception (odor quality). Moreover, processed signals are categorized in the primary olfactory cortex, however this categorization is more related to odor quality[45]. These physiological differences between olfaction and other senses suggest that, although other senses also induce numerous cognitive phenomena, olfaction can distinguish objects more objectively. However, in the case of olfaction, this is a difficult issue even with physiological evidence, which will be discussed in a later session.

## 2 Upstream stages of olfactory processing

Olfaction is the process of the perception of smell from volatile chemicals. From a physiological standpoint, perceiving odor begins with activating a specific olfactory receptor (OR) repertoire set in response to volatile chemicals[35,62,83]. During inhalation, volatile chemicals come from the nose and attach in the mucosa of the olfactory epithelium (OE) which is located in the roof of our nasal cavity. There are about 350 to 400 types of olfactory sensory neurons (OSNs) embedded in the mucosa. OSNs are bipolar neurons that extend an axon to both the olfactory bulb and the ciliary membrane that contains the OR. OR are activated by volatile chemicals via a biochemical transduction cascade (Figure 3). Once the cascade begins, chemicals induce activation in OSN and these patterns can lead to a remarkable number of odors (numbering in the thousands) that can be discriminated (Figure 4). Once an OSN is activated the signal is processed by the olfactory bulb (OB).

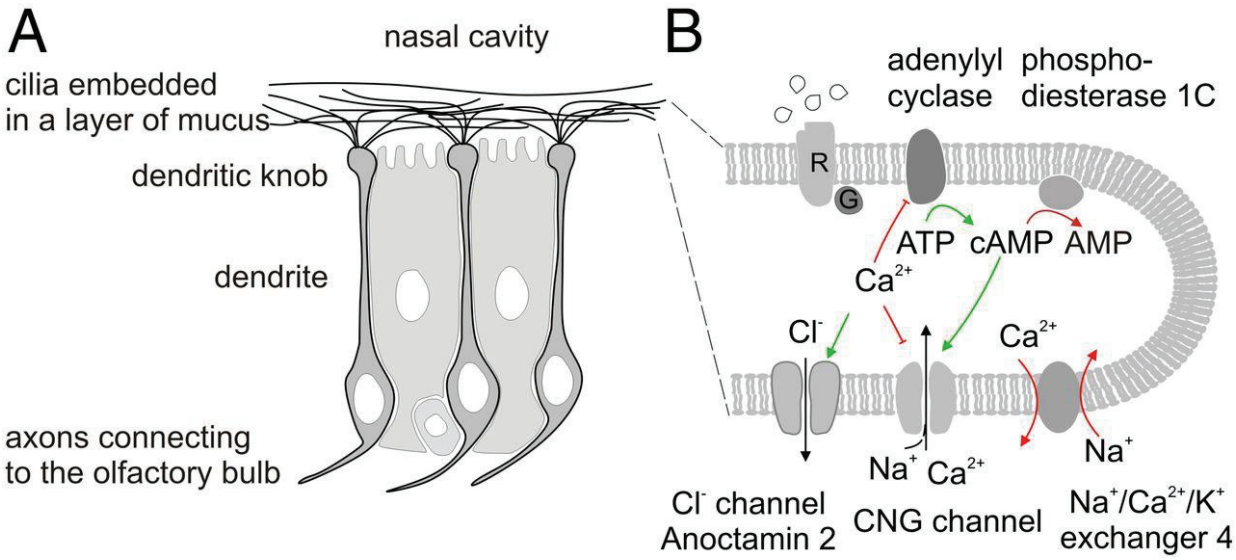



**Figure 3. Schematic of the olfactory epithelium with OSNs and the transduction pathway of the olfactory receptor (from Reisert et al., 2019) [101].** A. Schematic of the OSNs. B. Biochemical transduction cascade via olfactory receptors. Odorants activate odorant receptors, initiating a transduction cascade that depolarized the OSNs.

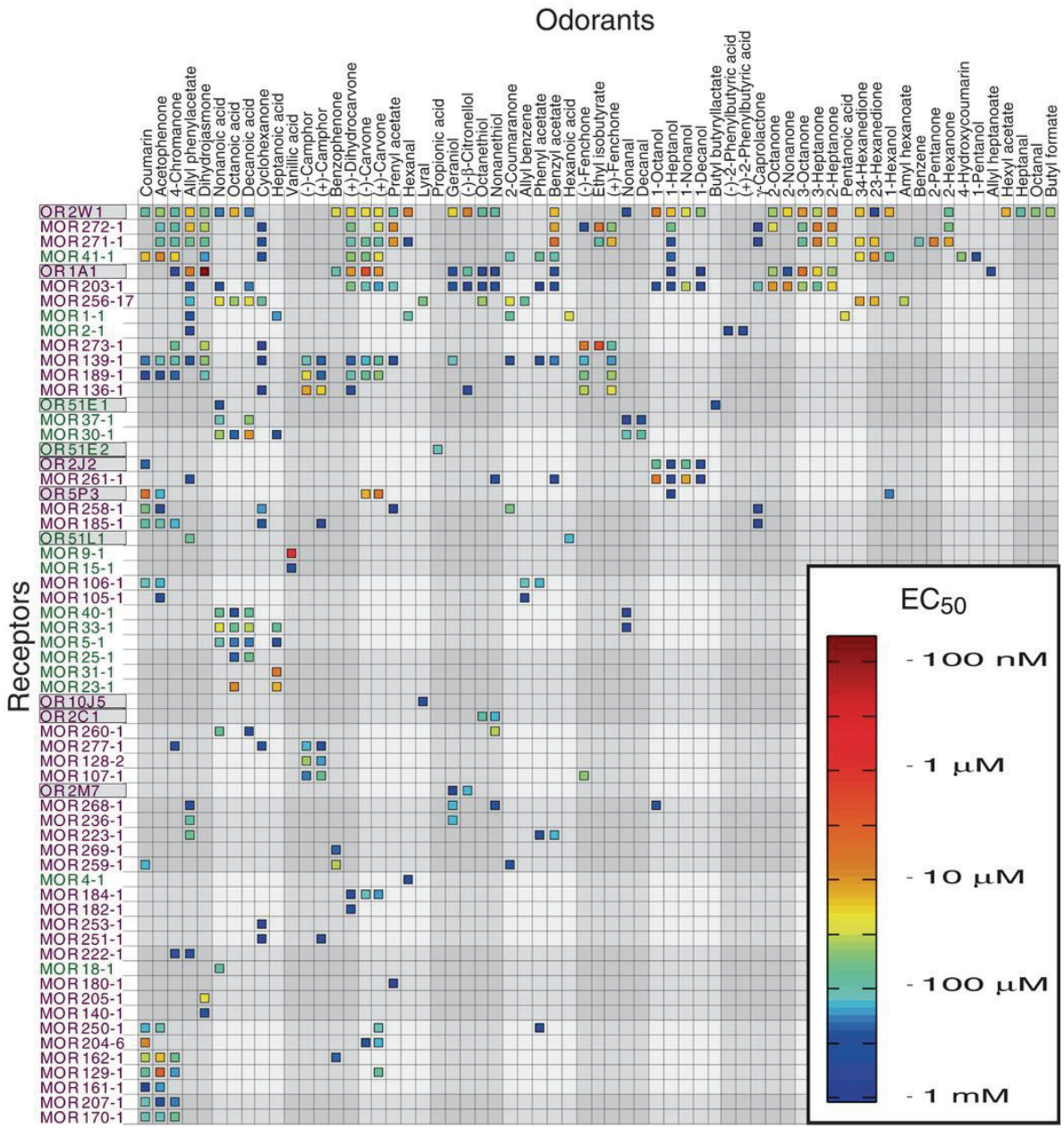



Figure 4. Odor coding by odorant receptor repertoire [105].

The OB is known to play a role in discriminating odors and encoding odor information[83,103]. The OB has a multi-layered structure and consists of a glomerular layer, external plexiform layer, mitral cell layer, internal plexiform layer, and granule cell layer. The axon of the olfactory sensory neuron extends to the olfactory bulb, which forms a synapse with the glomeruli present in the glomerular layer (Figure 5). A characteristic part of the olfactory bulb is that the olfactory stimuli transmitted from the olfactory bulb are aligned according to the type of olfactory receptors, and the axon of one olfactory sensory neuron makes a synaptic connection with only one glomerulus. In addition, the mitral cells and tuft cells connected to the olfactory cortex and the periglomerular interneuron association nerves surrounding the glomerulus also form synaptic connections [63]. Animal studies have shown that the ORNs map to specific locations in the OB glomerular layer. This is a similar mapping as vision, where specific receptors map to specific areas in the primary visual cortex. However, the exact structural organization of the OB in humans is not yet known [43]. While the signals from the OSN are processed in the OB, signals of physical–chemical properties from odor are processed in the glomerular layer [21]. Although the olfactory signal from OE corresponds to a segregated map in the OB, spatial segregation disappears in the piriform cortex (one of the primary olfactory areas) and returns to a highly distributed organization in which different odorants activate unique but dispersed ensembles of cortical neurons [53,120]. These findings suggest that the physical–chemical properties of odor translate to other properties in the OB. From here olfactory signals are transferred in parallel to the primary olfactory areas (e.g., piriform cortex, amygdala, and entorhinal cortex) with bidirectional connections. Secondary olfactory areas include the orbitofrontal cortex (OFC) and hippocampus [45].

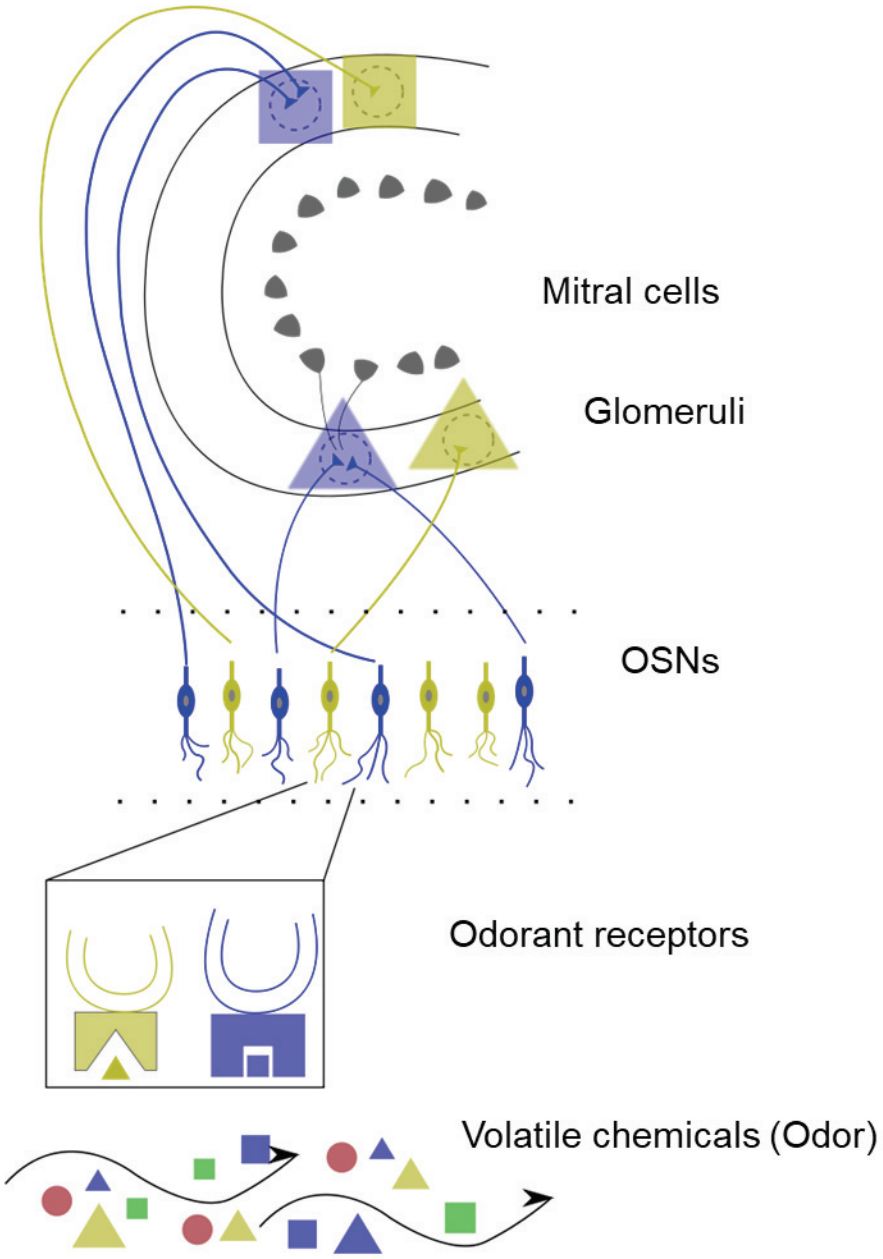



**Figure 5. Modified schematic from earlier proposed pathways of the olfactory system that included the olfactory epithelium and bulb (modified from Auffarth et al., 2011[4]).**

## 3 Olfactory processing in the brain

During olfaction, odor object perception begins in the OE and is eventually processed by the brain. Axonal projections from the mitral and tufted cells in the OB are connected via the olfactory tract to several areas including the anterior olfactory nucleus, olfactory tubercle, piriform cortex, amygdala, and rostral entorhinal cortex where information is subsequently processed (Figure 6, Figure 7). These areas are collectively known as the 'primary olfactory cortex'. The human piriform cortex is specifically known to process information related to odor categorization. Previous evidence suggests that the piriform cortex is activated during learning, memory, and motivational and cognitive states[48,78] in addition to its role in processing odor information. Interestingly, in the well-known patient H.M who underwent resection of the bilateral temporal cortex also demonstrated normal odor detection function, though judgments of similarity between pairs of odors were impaired [36]. This evidence suggests that the temporal lobes where the piriform cortex is located are crucial for processing information about odor object quality. As previously mentioned, the piriform cortex does not preserve topographically ordered spatial maps from the OB. However, fMRI studies of the human piriform cortex suggest a relationship between odor object quality perception and spatially organized neuronal patterns which are sufficient to represent odor quality [44,45,65,75,99,115,120,141]. Temporal patterns are also important as studies found that neurons varied across olfactory stimuli in their magnitude of spike firing and temporal characteristics (including spike latency, duration) [102]. Although there are limited studies regarding temporal patterns in the human piriform cortex, Jiang et al., (2017) suggest that theta oscillations convey odor-specific content in the human piriform cortex [56].

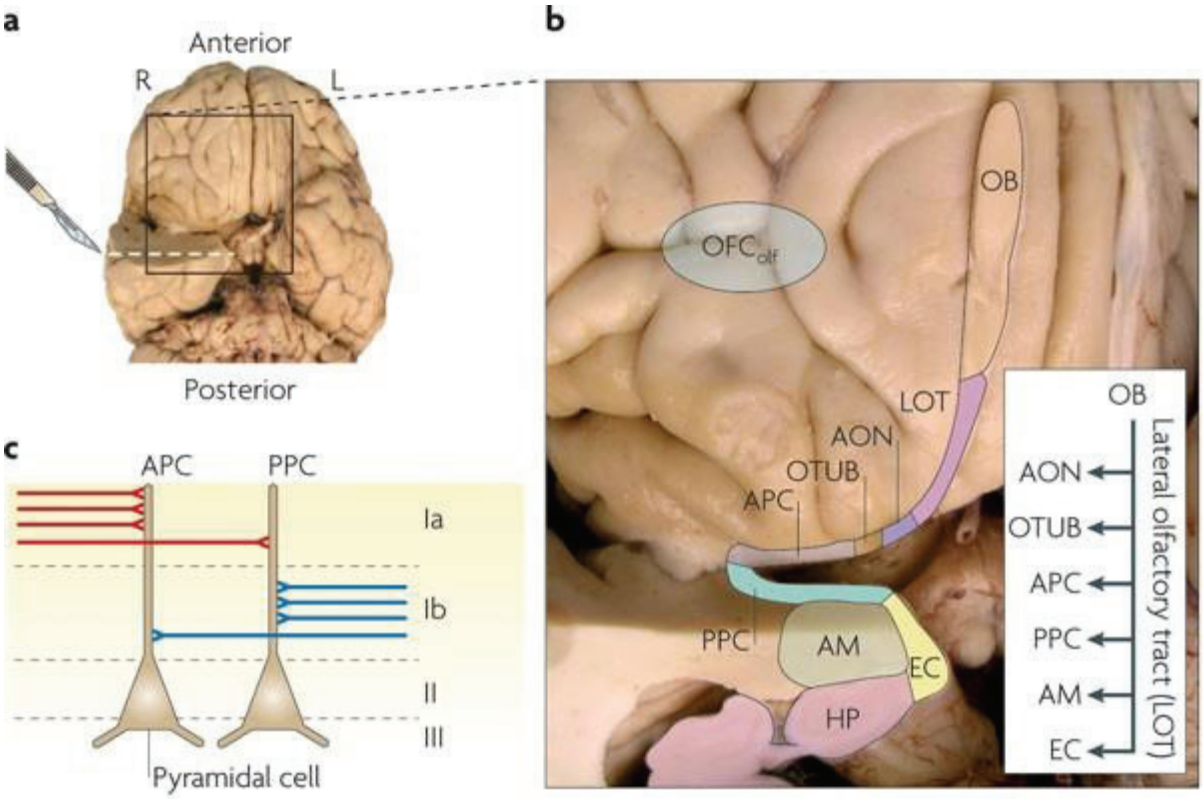



**Figure 6. Anatomy of the human olfactory pathway in the brain (from Gottfried, 2010[45])** A. A ventral view of the human brain. B. Lateral olfactory tract. Signal output from the olfactory bulb (OB) transfers through the lateral olfactory tract (LOT) and is subsequently processed in numerous regions, including the anterior olfactory nucleus (AON), olfactory tubercle (OTUB), anterior piriform cortex (APC), posterior piriform cortex (PPC), amygdala (AM), and entorhinal cortex (EC). Downstream processing targets also include the hippocampus (HP) and orbitofrontal cortex (OFC). C. Schematic of the synaptic organization of the piriform cortex. Pyramidal neurons are located in cell body layers II and III, and their apical dendrites project to molecular layer I. Layer I is divided into a superficial layer (Ia) which receives afferent signals from the OB (shown in red) and a deeper layer (Ib) which receives afferent signals from other areas of the primary olfactory cortex or higher-order regions of the brain (shown in blue).

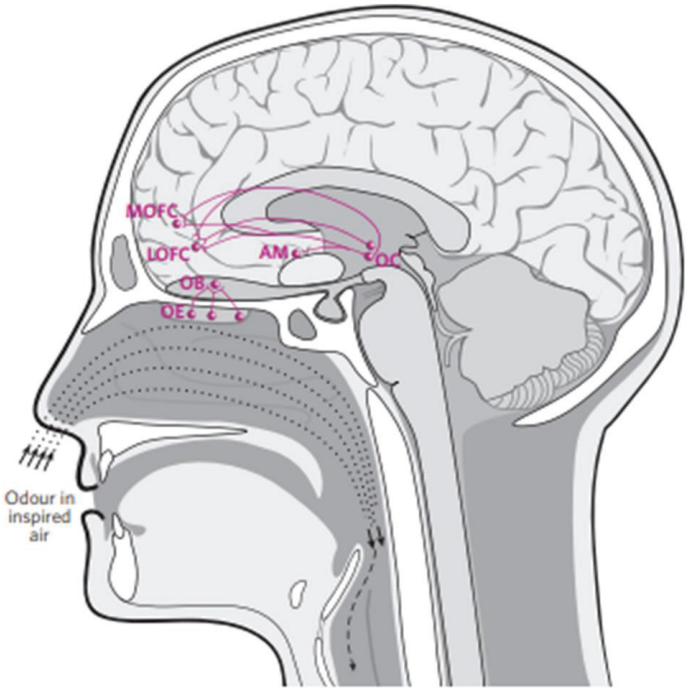



**Figure 7. Olfactory pathways in the human brain (modified from Shepherd, 2006[111]).** AM, amygdala; LOFC, lateral orbitofrontal cortex; MOFC, medial orbitofrontal cortex; OB, olfactory bulb; OC, olfactory cortex; OE, olfactory epithelium.

The OB is also directly connected with other areas such as the olfactory tubercle and amygdala, though there are few findings that suggest olfactory object processing plays other roles. The olfactory tubercle may be involved in the determination of the source of olfactory information [136] and emotional processing [139]. The amygdala may represent odor intensity and valence [3,57], though studies combined fMRI signals across subregions. Intracranial EEG (iEEG) studies similarly suggest that the amygdala is involved in odor coding [50,56].

Olfactory signals relay from the primary olfactory cortex to the secondary olfactory in areas such as the OFC, perirhinal cortex, and hippocampus. Interestingly, monosynaptic projections from the piriform cortex to the OFC ensure that odor information bypasses the thalamus which is a target for all other sensory modalities [19]. The OFC is also well known as an olfactory-related area that processes olfactory information [107,122]. Although non-mammalian vertebrates interact with the olfactory environment without the OFC, in the case of more complex animals, the odor-evoked activity is processed in the OB and OFC [122]. In humans, patients with OFC lesions have difficulties with odor discrimination, identification, and memory [58,100,135]. Taken together, these findings suggest that the OFC may interact with higher-odor olfactory functions.

## 4 Odor object quality perception

As previously mentioned, one odor chemical can induce activation in several OSNs. Similarly, one odor chemical can induce a multidimensional axis of odor perception, though there is less evidence for perceptual space [20,41]. However, increasing evidence of odor discrimination ability in humans suggests that humans exhibit high performance in categorizing odors, despite possessing low performance in identifying and naming those odors [16,73,77,133].

Humans have good odor discrimination performance as illustrated in Figure 8. Humans can discriminate between two odorants of different concentrations as low as 7% [16] and even smaller changes of a component in a mixture can be recognized[77]. Humans can also discriminate the smallest alterations in molecular structure, such as between odorants equal in the number of carbons but differing in functional group [73]. In contrast, humans have low odor identification ability as indicated in tests of naming performance (Figure 9). According to previous studies, humans cannot name more than 50% of odorous household items that they use every day [17,76].

Odor discrimination and identification performance change based on increased exposure to a smell [55,128]. Higher familiarity decreases discrimination errors of initially unfamiliar odors, and odor habituation in the rat piriform cortex has been shown to be the basis for the distinction between component and binary odor mixtures [129].

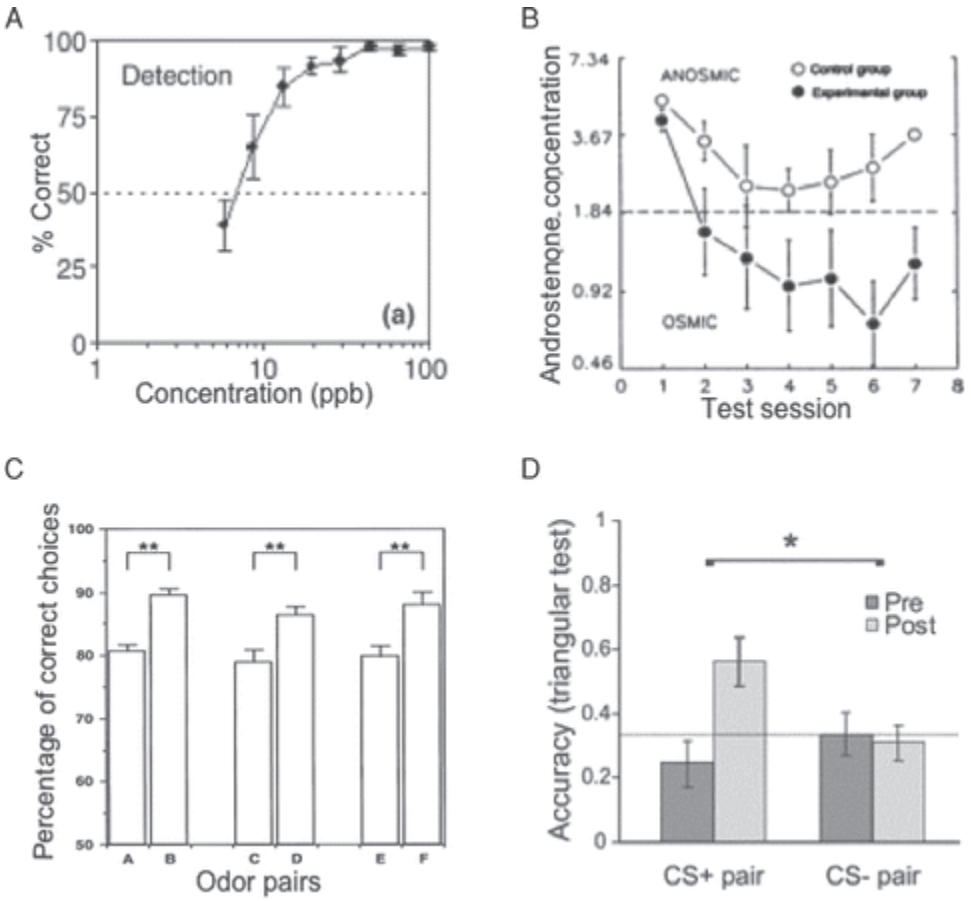



**Figure 8. Performance of humans in odor detection and discrimination (from Yeshurn & Sobel, 2010[133]).** A. Accurate detection of ozone corrected for guessing (averages ± SEM) is significantly beyond the 50% chance level by ~10 parts per billion (ppb) B. Androstenone detection thresholds decreased in an exposed group, but not in an unexposed group. The dashed line represents the functional definition of androstenone anosmia (adapted from Wysocki et al. 1989). C. Discrimination between similar odorants: column A, odor pairs that involved two hexenols; column B, odor pairs that involved a hexanol and a hexenol; column C, odor pairs that involved two cis-hexenols; column D, odor pairs that involved two trans-hexenols; column E, odor pairs that involved hexenols sharing the same geometry but differing in the position of the double bond by only one carbon atom; and column F, odor pairs that involved hexenols sharing the same geometry but differing in the position of the double bond by two carbon atoms (adapted from Laska 2005). D. Odor discrimination accuracy was at chance (dashed line) for CS+ and CS− pairs before conditioning, but selectively improved for the CS+ pair after conditioning. Error bars, ±SEM (adapted from Li et al. 2008)

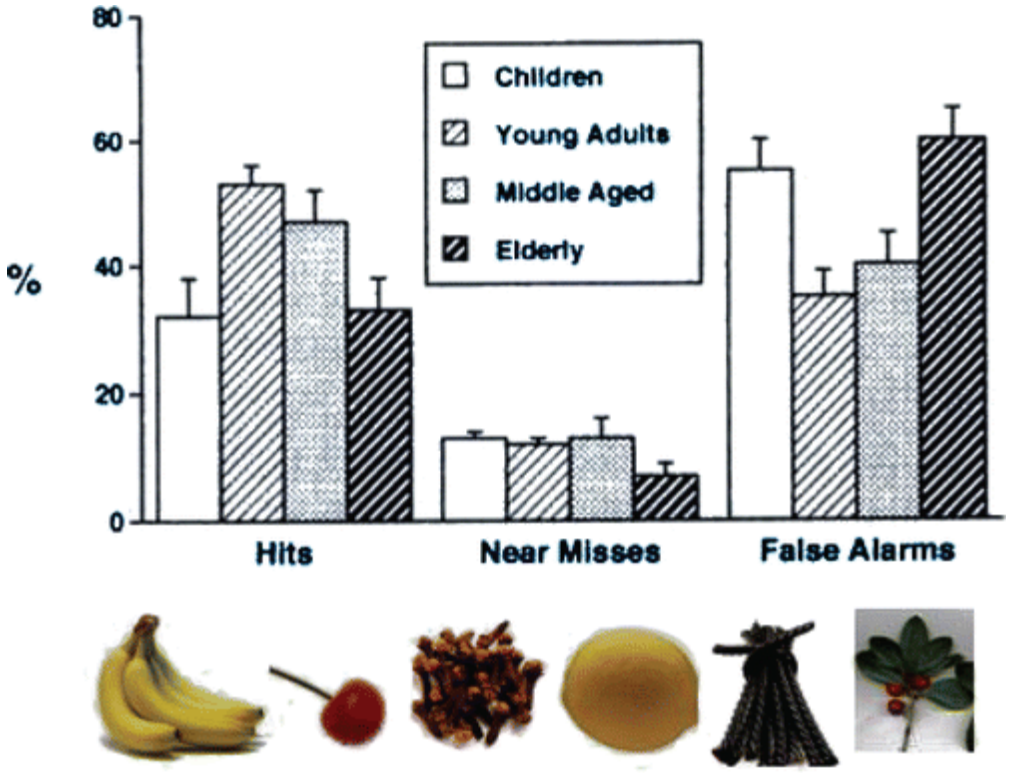



**Figure 9. Human odor identification and naming (from Yeshurn & Sobel, 2010[133]).** Percent correct (hits), near-miss, and far-miss (false alarms) identification (SEM) for children, young adults, middle-aged, and elderly persons (from de Wijk & Cain, 1994a). Images represent the odor sources used in this experiment: banana, cherry, cloves, lemon, licorice, and wintergreen.

## 5 Temporal view of olfactory processing

Odor quality is processed by the brain in many steps (Figure 10). As previously mentioned, during olfaction volatile chemicals stimulate the olfactory epithelium and are eventually processed by the brain [44,45,65,75,99,115,120,141]. The OB and piriform cortex may play essential roles in categorizing odor, and the orbitofrontal cortex may categorize odor by higher processing such as odor identification. However, though numerous studies have been conducted, the temporal view of olfactory processing is limited in human studies.

As highlighted in the introduction session, our nerves use both spatial and temporal activation patterns to encode information. In the case of the olfactory system, mitral/tufted cells in the OB of mice have a first-spike latency relative to the start of a sniffing action which seems to encode much of the information about an odor [130]. This temporally structured odor information transfers to the piriform cortex where odor is encoded [119]. The temporal pattern of the human piriform cortex reflects theta oscillations which may represent the processing of specific olfactory information [56].

To understand functions in the brain, an understanding of which brain 'areas' become activated 'when' is important. Most brain areas are bidirectional pathways that can be modulated both up and downstream of other brain areas. For example, satiety by food influences neuronal activity in the OFC [104], and aversive learning increases piriform cortex discrimination with the same behavior output. These findings suggest that during olfaction, our brain is activated dynamically rather than sequentially.

Studies have examined a temporal view of the processing of olfactory information (Figure 11). When odor stimulates our olfactory system, previous studies have suggested that odor identification can be measured at around 400ms [72]. Moreover, odor-induced changes in human sniffing behavior were observed before 160ms [68]. These behavioral studies suggest that humans can perceived odor within at least 400 ms and some odor signals are processed within 160 ms in primary olfactory areas.

Recent studies of brain waves activity (EEG and MEG) demonstrate that olfactory signals can reach the PC within approximately 40 ms[87,117] . Additionally, direct electrical signal data from studies of the human epileptic brain suggest that the PC and OFC process odor information earlier than 200ms [56]. Although prior studies did not focus on odor categorization, they suggest a spatiotemporal pattern of

neuronal firing during odor processing. In the case of event related potential (ERP) studies in olfaction, the temporal issue is quite controversial. Results were based on previous studies of OSNs from the OE which indicated that odor signal processing by OSNs was at approximately 300ms [39,95]therefore they focused on odor signaling after 200ms [27,40,52,106].

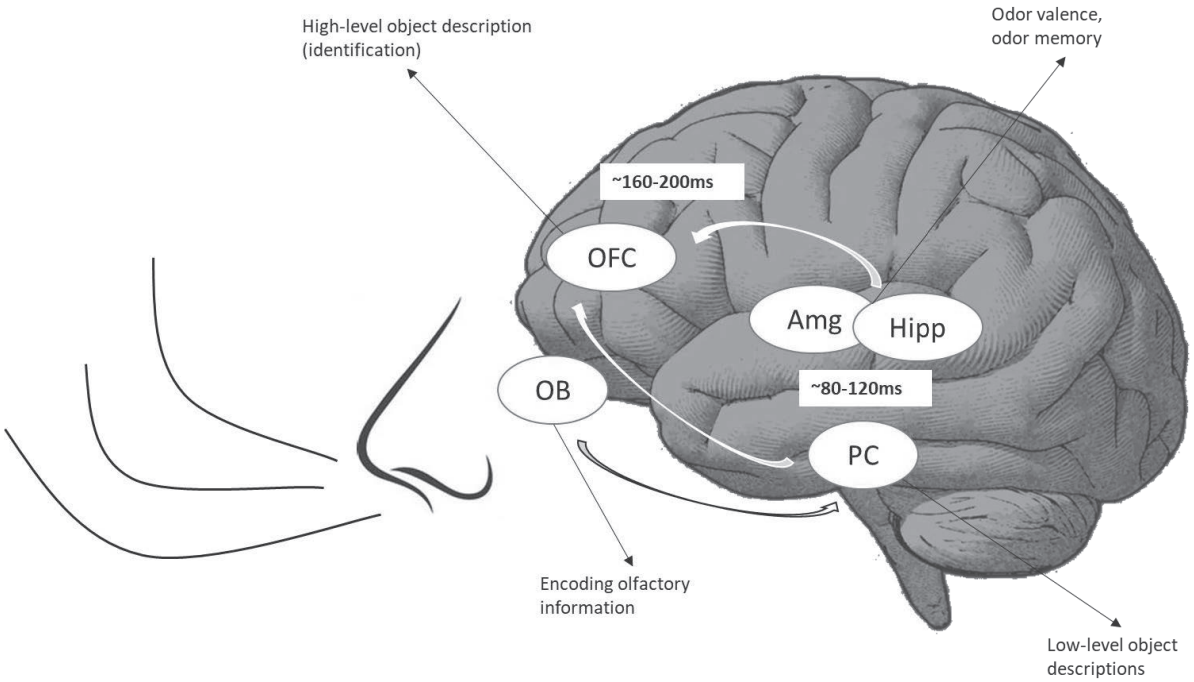



**Figure 10. Spatiotemporal view of olfactory processing.** Amg: amygdala; Hipp: hippochampus; OB: olfactory bulb; OFC: orbitofrontal cortex.

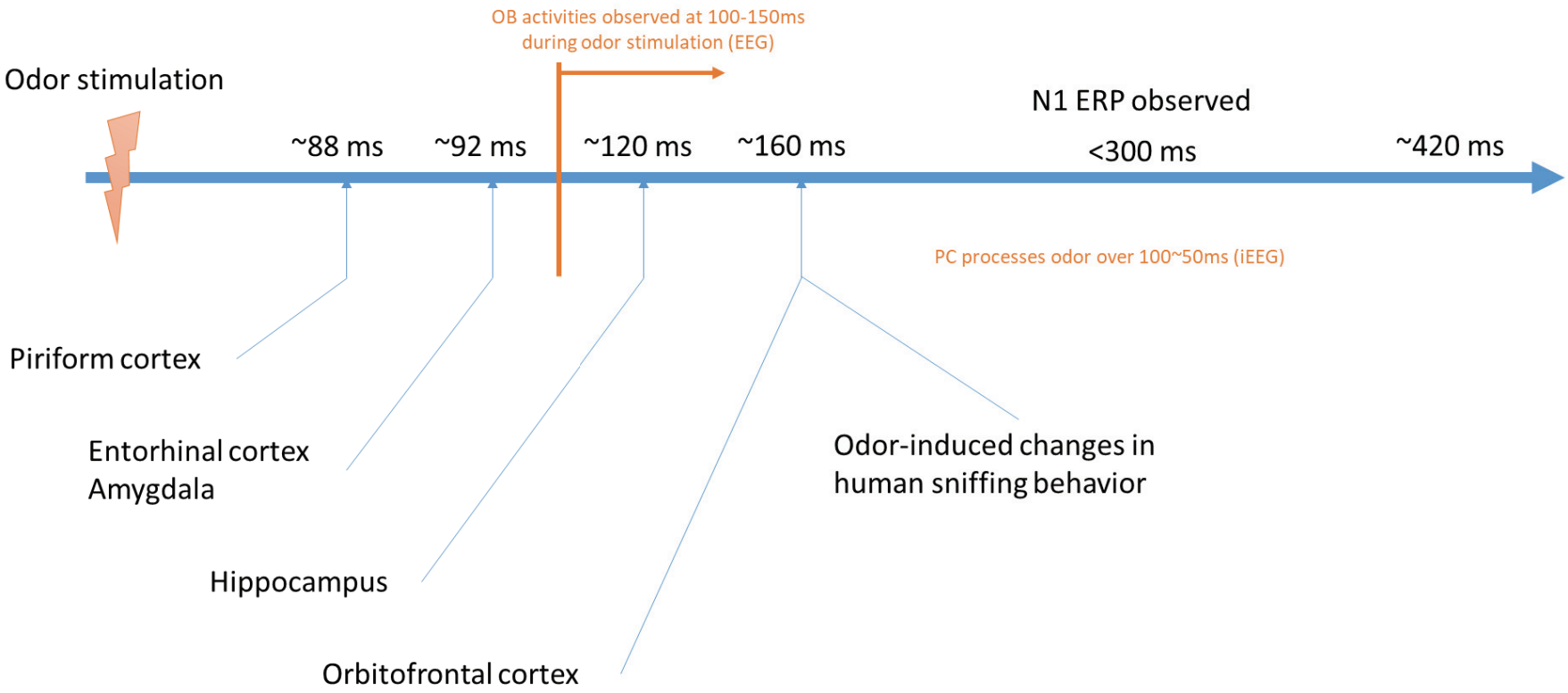



**Figure 11. Schematic diagram of temporal view of olfactory processing in human brain.** Olfactory related areas are activated within 200ms. In the case of ERP studies, most focused on signals occurring after 300ms.

## 6 Use of EEG in the current study

Electroencephalography (EEG) was chosen as a brain imaging tool in the current study for various reasons. Primarily EEG has previously been used as a high temporal resolution technique to study neurocognitive processes [25] including olfaction as EEG captures dynamic cognitive events in the time frame in which cognition occurs (Figure 12). Both cognitive processes and processes in the olfactory system occur rapidly, requiring a dynamic tool like EEG. The second reason is that EEG is useful for studying neurocognitive processes as it measures direct neural activity [15]. EEG measures voltage fluctuations which reflect the activity of populations of neurons. Moreover, oscillations in brain waves that can be observed using EEG are direct reflections of neuronal oscillations in the brain. This can be an advantage as blood-oxygen-level-dependent (BOLD) signal based measurement has a more complex relationship between rear neural activities and BOLD signal [112]. Third, EEG provides multidimensional information by measuring voltage change over time and space and includes information regarding frequency, power, and phase as well. The multidimensionality of EEG data allows for analysis inspired by known physiological mechanisms. This provides an opportunity to link human and non-human animal neural activity studies [25].

. One of the defects of using EEG is that it is a less effective tool for measuring deep brain structures. Although several studies suggest EEG is capable of measuring activity from deep brain structures [85], both MEG and iEEG are better techniques to address this issue. Though both MEG and iEEG are similar methods that would provide advantages in the current study, there are also drawbacks to these techniques. MEG measures physiological properties similar to EEG; however, different results can be produced. EEG can detect both radial and tangential sources. In contrast, MEG is maximally sensitive to tangential sources but is poor in detecting radial sources. Previous studies suggest that EEG and MEG show different midfrontal theta performance and that EEG is easier to measure than MEG as the theta waves may be evoked from radial dipoles [116,118]. The theta frequency band is a focus in this study, therefore EEG would be more appropriate rather than MEG. Similarly, iEEG is a powerful tool because it can provide a good signal with specificity for location. However, this tool has limited accessibility. With the exception of well-trained researchers, limited hospital and investigators are trained in use of iEEG. Moreover, iEEG is typically reserved for clinical use such as in cases of epilepsy. Several studies

suggest that olfactory dysfunction can be observed in epilepsy patients where olfactory regions are impacted [38]. For all the above reasons, EEG was chosen as the best option for the current study.

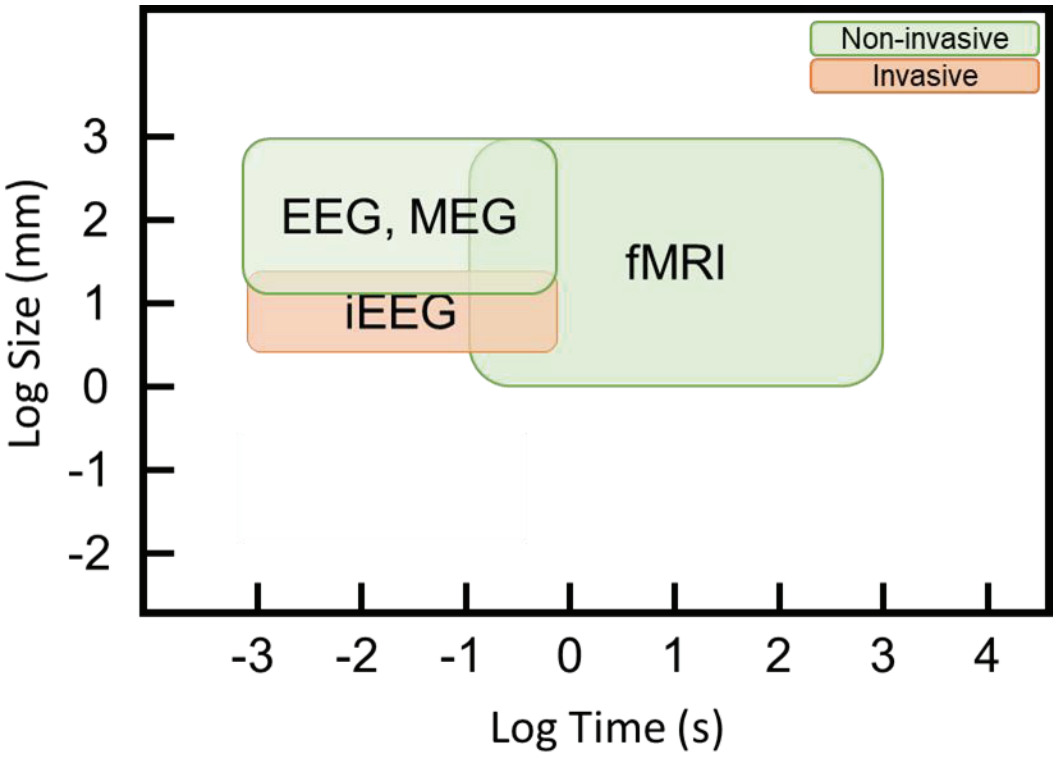



**Figure 12. Temporal and spatial resolution of brain imaging devices.** EEG: spatial resolution (7-10 mm), temporal resolution (<1 ms), MEG: spatial resolution (2-6 mm), temporal resolution (< 1 ms), fMRI: spatial resolution (1-2 mm), temporal resolution (1s).

# III. Aims of the thesis

The aim of the current thesis is to understand odor information processing in the human brain through temporal activation patterns by using EEG. As mentioned in the introduction section, there is limited research that defines odor similarities or differences which raised the need for increased study in this area. Current research has established that activating the specific OR repertoire set in response to volatile chemicals induces the basic logic of odor differences. This information is modulated and encoded by the OB and eventually processed in areas of the brain including the PC and OFC. Although neural information coded by both spatial and temporal patterns of activity exists, research regarding the timing of odor processing steps and temporal patterns remains limited. Thus, the current study focused on olfactory information processing in the human brain using EEG.

Three studies were designed to address this question. First, the possibility that direct olfactory processing can be measured by EEG signal at an early time point was established. Although temporal studies are important, this information can be obtained using proper methods and observation. Verifying direct olfactory signals at an early time point is important before establishing the temporal settings for the experiment. Secondly, the current study examined temporal patterns during olfaction using similar and dissimilar odors. By using multivariate analysis, I focused on when odor categorization occurred in the human brain. Lastly, odor similarity was categorized based on the multidimensional axis of odor object quality. The final output may differ depending on how the brain categorizes odor and the precision of these measurements. Thus, the current study focused on evaluating odor similarity based on surveys and behavioral results.

# IV. Materials and Methods

## 1 General methods

### 1.1 Participants

The participants in the current studies included young adults who were predominantly undergraduate and graduate students (age range: 18 to 33). All participants were right-handed, displayed normal olfactory functions (examined by Sniffin' sticks test), and had no history of psychological or neurological disease. The participants were recruited through bulletin board ads, e-mails to student lists, and use of online social media (Facebook).

For study 1, 14 participants (6 males, 8 females, mean age 21.1 years [SD=2.4]) were recruited from the Daegu Gyeongbuk Institute of Science and Technology. Among them, only 13 participants were included as data from one male participant was excluded due to technical problems.

For study 2, 24 participants (15 females, 9 males; mean age 19 years [SD= 2.37] from Daegu Gyeongbuk Institute of Science and Technology were included.

For study 3, 96 participants (32 females, 64 males; mean age 21.02 years [SD = 2.54]) from Daegu Gyeongbuk Institute of Science and Technology were included. Participants were randomly divided into three groups (Table 8). Participants in group 1 (n = 32, 14 females, 18 males) were subjected to stimulation by Isovaleric acid and heptanol with a blank screen. Participants in group 2 (n = 32, 10 females, 22 males) were subjected to stimulation by IVA with the "cheese" verbal cue on the screen. Participants in group 3 (n = 32, 8 females, 24 males) were subjected to stimulation by IVA with the "vomit" verbal cue on the screen. Divided groups did not differ significantly with respect to age, odor threshold, or odor discrimination based on results of a one-way ANOVA. For the second experiment, participants were recruited approximately one month later. Participants in group 1 (n = 16, 4 females, 12 males) were subjected to stimulation by IVA with the "cheese" verbal cue on the screen. Those in

group 2 (n = 16, 5 females, 11 males) were subjected to stimulation by IVA with the “vomit” verbal cue on the screen. In group 3 (n = 16, 7 females, 9 males) participants were subjected to stimulation by IVA and Hep with a blank screen.

### 1.2 Ethics

All studies in this thesis received approval from the Institutional Review Board ethics committee of Daegu Gyeongbuk Institute of Science & Technology (https://larc.dgist.ac.kr). I followed the ethical principles as stated in the Declaration of Helsinki. All participants were fully informed about the potential risks of materials and procedures. I also informed them that they had the right to quit at any time during the experiment. Participants were included in the study after giving informed consent.

### 1.3 Sniffin’ sticks test

The Sniffin’ sticks test was used to examine the olfactory functions of participants (Figure 13). I used the Sniffin’ sticks kit from ‘Burghart sniffin’ sticks’ (company: Medisense). Olfactory threshold, discrimination, and identification were tested using this kit. Participants were instructed to close their eyes or wear an eye patch during testing. During the test, a short break for 20-30 seconds was taken to avoid odor adaptation and stress. Each pen was presented only once for 3 to 4 seconds approximately 2 cm from the edge of the nostril.

For the odor threshold test, I used a kit that consisted of 16 triplets of pens (total of 48 pens) with numbers from 1 to 16. The three pens in each triplet are distinguished by the color of their cap: red, green, and blue, and red pens are impregnated butan-1-ol diluted in a solvent that decreased in concentration. An interval of at least 3 seconds occurred between presentations of pens. Participants were presented with successive triplets of pens with increasing butan-1-ol concentrations (decreasing numbers) until the first correct response was obtained. When participants answered with a correct response twice within a concentration level, the level was designated as the threshold score.

For the odor discrimination test, 16 triplets of pens were again presented, with numbers from 1 to 16. The blue and red pens of each triplet were impregnated with the same odor and the third (green) pen

was impregnated with a different odor. The subject is required to identify which pen of the triplet has a different odor from the other two. The pens are presented in different orders, as previously described. The discrimination score corresponded to the number of correct responses out of sixteen.

For the odor identification test, 16 blue pens with black numbers were presented. Each pen is presented only once, with an interval of at least 30 seconds at which time participants must choose an answer from a list of four choices. The identification score corresponded to the number of correct responses.

**Figure 13. The Sniffin' Sticks test kit (Burghardt®, Wedel, Germany)**

## 2 Stimuli

### 2.1 Odor preparation

In study 1 used 1-heptanol (Sigma-Aldrich, LOT#STBD9537V) and 2-acetylpyrazine (Sigma-Aldrich, Lot #MKCB169V). These odors were chosen as they are structurally and perceptually different, which helps to avoid cross adaptation. The 1-heptanol was diluted in mineral oil (Sigma-Aldrich, LOT# MKBZ6778V) to 10% (v/v) to be aliquoted in 1.5 mL tubes and 20% concentration for use in a custom-built olfactometer. The 2-acetylpyrazine was diluted to 0.1% (w/v) in distilled water.

In study 2, 2-acetlypyrazine (Sigma-Aldrich, LOT#MKCB1629V), 2,3,5-trimethylpyrazine (Sigma-Aldrich, LOT#STBG6509), and hexan-1-al (Sigma-Aldrich, LOT#MKCC2925) were used (Figure 1-A). 2-acetlypyrazine and 2,3,5-trimethylpyrazine were each diluted in distilled water to a concentration of 0.1%. Hexan-1-al was diluted in poly-ethylene glycol (Sigma-Aldrich, LOT#BCBP4448V) to a concentration of 0.5%. Distilled water (DW) was used to establish baselines.

In study 3, Isovaleric acid (Sigma-Aldrich, LOT#STBG4549V) and Heptanol (Sigma-Aldrich, LOT#STBD9537V) were used. Isovaleric acid was diluted in mineral oil (Sigma Aldrich, LOT#MKBZ6778V) to a 0.01% final concentration. Heptanol was similarly diluted in mineral oil to a final concentration of 0.5%.

### 2.2 Odor delivery

In study 1, odors were delivered using blotting paper. Two hundred microliters of the diluted odor was used to treat the tip of blotting paper and these prepared blotting papers were sealed in 50 ml falcon tubes. Odors were stimulated during experiments by unsealing the falcon tubes. Participants were allowed to continue sniffing the odorant with over a 30 s interval between each sniff until they rated all 146 odor quality descriptors.

In the overall EEG experiment, odors were offered via the olfactometer. The olfactometer was linked to

a mask (without canula) which participants wore. All odors were delivered to the participant immediately after an inhalation peak which persisted until the next inhalation peak (approximately 2 s). When odors were offered by the olfactometer, the airflow speed was 5.18 m/s.

## 3 Survey

### 3.1 Odor quality response

To measure odor quality responses, I evaluated 146 odor descriptions using rating scales from 1 to 9 (Table 1). A score of 0 indicates an 'Unknown description'. For the odor quality description task, participants chose a suitable scale of odor quality descriptions from 146 odor descriptions[34] following odor stimulation.

### 3.2 General odor response

To measure additional odor responses, pleasantness, intensity, familiarity, edibility, and relaxing effect were evaluated using a rating scale of 1 to 9. Odor descriptions were recorded to define how participants identified odors (Table 2).

**Table 1. Questionnaires with 146 items from the Odor quality rating test.**

| Descriptor | Unknown Descriptor | Disagree | ..... | Neither agree nor disagree | ..... | Agree | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| alcohol-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| almond-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| animal | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| anise (licorice) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| apple (fruit) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| aromatic | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| bakery (fresh bread) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| banana-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| bark-like, birch bark | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| bean-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| beery (beer-like) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| bitter | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| black pepper-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| burnt candle | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| burnt milk | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| burnt rubber-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| burnt, smoky | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| buttery (fresh) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| cadaverous, like dead animal | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| camphor-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| cantaloupe, honey dew melon | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| caramel | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| caraway | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| cardboard-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| cat-urine-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| cedarwood-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| celery | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| chalky | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| cheesy | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| chemical | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| cherry (berry) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| chocolate | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| cinnamon | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| clove-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| coconut-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| coffee-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| cologne | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| cooked vegetables | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| cool, cooling | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| cork-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| creosote | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| crushed grass | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| crushed weeds | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| dill-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| dirty linen-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| disinfectant, carbolic | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| dry, powdery | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| eggy (fresh eggs) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| etherish, anaesthetic | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| eucalyptus | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| fecal (like manure) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| fermented (rotten) fruit | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| fishy | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| floral | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| fragrant | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| fresh green vegetables | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| fresh tobacco smoke | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| fried chicken | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| fruity (citrus) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| fruity (other) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| garlic, onion | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| geranium leaves | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| grainy (as in grain) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| grape-juice-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| grapefruit | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| green pepper | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| hay | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| heavy | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| herbal, green, cut grass | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| honey-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| household gas | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| incense | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| kerosene | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| kippery (smoked fish) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| laurel leaves | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| lavender | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| leather-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| lemon (fruit) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| light | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| like ammonia | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| like blood, raw meat | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| like burnt paper | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| like cleaning fluid (carbona) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| like gasoline, solvent | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| like mothballs | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| malty | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| maple (as in syrup) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| meaty (cooked, good) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| medicinal | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| metallic | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| minty, peppermint | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| molasses | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| mouse-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| mushroom-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| musk-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| musty, earthy, moldy | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| nail polish remover | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| nutty (walnut, etc.) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| oak wood, cognac-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| oily, fatty | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| orange (fruit) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| paint-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| peach (fruit) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| peanut butter | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| pear (fruit) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| perfumery | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| pineapple (fruit) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| popcorn | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| putrid, foul, decayed | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| raisins | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| rancid | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| raw cucumber-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| raw potato-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| rope-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| rose-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| rubbery (new rubber) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| sauerkraut-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| seasoning (for meat) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| seminal, sperm-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| sewer odor | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| sharp, pungent, acid | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| sickening | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| soapy | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| sooty | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| soupy | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| sour milk | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| sour | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| spicy | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| stale | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| stale tobacco smoke | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| strawberry-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| sulphidic | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Sweaty | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| sweet | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| tar-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| tea-leaves-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| turpentine (pine oil) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| urine-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| vanilla-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| varnish | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| violets | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| warm | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| wet paper-like | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| wet wool, wet dog | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| woody, resinous | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| yeasty | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

**Table 2. Odor identification task and questionnaires for additional odor responses.**

| | |
|---|---|
| Identify the odor | |

| | **Low** | | | ..... | | | | | **High** |
|---|---|---|---|---|---|---|---|---|---|
| Pleasantness | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Intensity | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Familiarity | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Edibility | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Relaxing effect | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

# 4 EEG

## 4.1 Electroencephalogram (EEG) Recording

Brain activity was monitored via EEG during the odor stimulation test. EEG signals were digitized via an EEG amplifier (ActiveTwo, BioSemi, Amsterdam, the Netherlands) and recorded with Ag/AgCl scalp electrodes from 64 positions of the international 10/20 system (Fp1, AF7, AF3, F1, F3, F5, F7, FT7, FC5, FC3, FC1, C1, C3, C5, T7 (T3), TP7, CP5, CP3, CP1, P1, P3, P5, P7, P9, PO7, PO3, O1, Iz (inion), Oz, POz, Pz, CPz, Fpz, Fp2, AF8, AF4, Afz, Fz, F2, F4, F6, F8, FT8, FC6, FC4, FC2, FCz, Cz, C2, C4, C6, T8 (T4), TP8, CP6, CP4, CP2, P2, P4, P6, P8, P10, PO8, PO4, and O2) on a BioSemi headcap (64 ch, BioSemi). Eye blinks (electrooculographic signals) were measured at approximately 2 cm above the outer canthus of the right eye. The sampling rate was 2,048 Hz, and the signals were analog-filtered via a 0.15 high-pass filter and a 2,048 Hz low-pass filter. A conductive electrolyte gel was used for a stable connection between the scalp and the electrodes. The impedance of each electrode was below 10kΩ. The electrophysiological activity was referenced to the common average of all channels.

## 4.2 Experimental environment during EEG recording

Odor stimuli were presented during inspiration. Before stimuli, a white cross was displayed on the LCD monitor; a blank screen (i.e., black background) was displayed during odor stimulation. Room humidity was set at 30-60% and room temperature was set at 21-25°C to ensure fixed vapor pressure of odor. The experimental room was fitted with soundproof walls and ventilation facilities to reduce noise and limit unexpected sensory stimuli.

### 4.3 ERP preprocessing

EEG data were downsampled from 2,048 Hz to 512 Hz. An offline bandpass filter was applied at 1–20Hz to minimize noises caused by muscle artifacts and skin potential. EEG data were segmented into epochs. Each epoch had a 500ms pre-stimulus period and a 1,500ms post-stimulus period. Starting stimulus points were set at the participant's exhalation peaks in every trial as an exhalation peak was a turning point to start inhalation. Before averaging epochs of conditions, filtered EEG data contaminated with eye blinks (>40 μV) or mismatched between the respiration cycle and odor offering time were discarded. Data from –500 to 0 ms in each epoch was used for baseline correction.

After EEG pre-processing, over 23 trials of EEG data per condition were averaged. Therefore, each participant had three ERP datasets under the three conditions ("none", "different", and "same"). According to previous studies, NP and PP peaks were determined as the most negative and the most positive peaks, respectively, between 40 ms and 200 ms in the ERP data of each participant [68,87,117]. For comparisons with negative and positive peaks [40], the most negative peak between 200 ms and 700 ms (N1) and the most positive peak between 300 ms and 800 ms (P2) were selected. In the grand average, each participant's ERPs were fitted based on negative potential peaks. The time points of NPs in the grand average were fitted based on the mean latency of all participants' negative potentials in each condition (Figure 14).

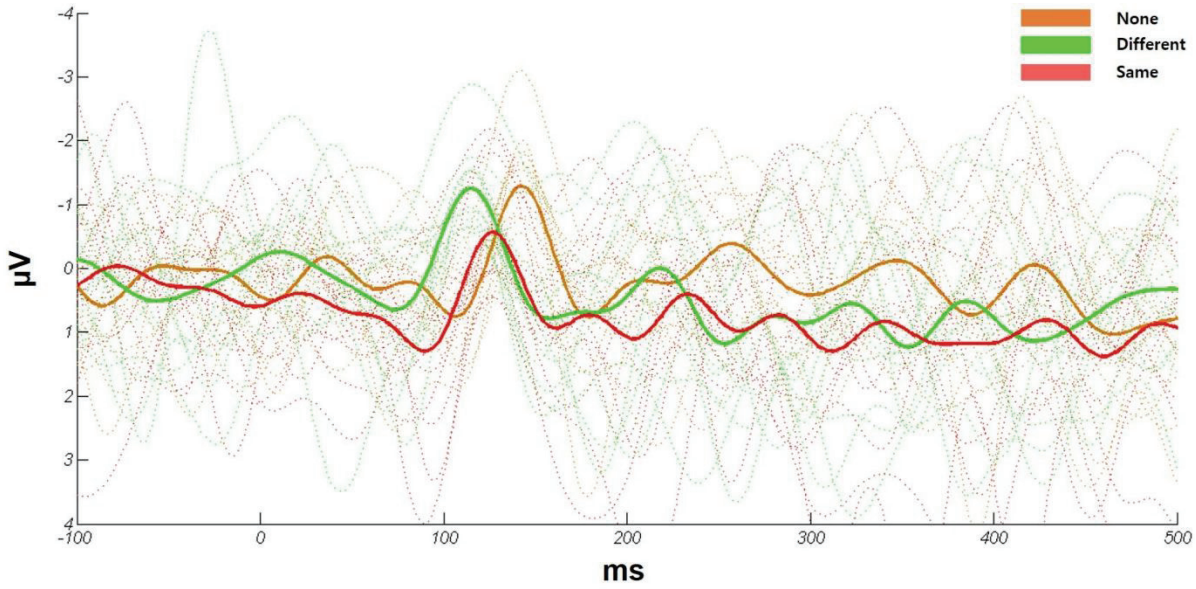



**Figure 14. Grand average event-related potential (ERP) across all participants (n = 13) under the "none", "different" and "same" conditions.** Dotted lines show the ERPs of individual participants. Thick lines are means of all participants under the three conditions. Mean ERP of odors under each condition from the C6 channel is shown.

### 4.4 EEG preprocessing

Data were first downsampled to 512 Hz. Next, each of the 64 EEG channels was filtered offline with high- and low-pass filters set to 0.5 and 50 Hz to exclude noise caused by skin potential, DC component of the amplifier, and muscle artifact. Data were segmented into epochs. The segment length was −500 to 1000ms. The filtered EEG channels were referenced to an average of total electrodes. Individual subject data were visually inspected for eye and muscle artifacts prior to artifact removal via automatic artifact rejection. The automatic artifact rejection procedure is as follows:

① Epoch rejection: epochs were rejected if their amplitude falls outside of ranges of -50 to 50 μV after ocular artifact correction [46].

② Channel rejection: channels were rejected if remaining epochs are less than 25 trials after epoch rejection.

③ Recording rejection: an EEG recording was rejected if more than 3 channels had to be rejected.

### 4.5 EEG data extraction for analysis

For the multivariate analyses in study 2, data were extracted from raw EEG data sets. For the time-variant data, event-related spectral potentials (ERSP) were extracted from each of the 64 electrodes. For ERSPs, the wavelet transform was used by EEGLAB[31]. Log-spaced 100 frequencies ranging from 0.5 to 50 Hz were calculated starting from -220ms to 720ms (200 time points). The wavelet cycle used 1 and 25 cycles as the lowest and highest frequencies, respectively. The baseline was set as the entire pre-stimulation period before 0 ms. DW was used for baselining to reduce olfactory-related signals. I divided ERSP into four data sets depending on the following frequency bands: 4-8 Hz (theta), 8–13 Hz (alpha), 13–30 Hz (beta), and 30–50 Hz (gamma). Next, I divided each data set again into eight sets based on time: 0-50 ms, 50-100 ms, 100-150 ms, 150-200 ms, 200-250 ms, 250-300 ms, 300-350 ms, 350-400 ms. Each data set was averaged by frequency before multivariate analysis. Therefore, extracted data contain the averaged value of each frequency band for each time point and electrode.

$$f_{CHn-Tm}[l] = \frac{1}{N}\sum_{l}^{l+N-1} ERSP_{CHn-Tm}[l] \quad (1)$$

***Tm*: ERSP time point**

***CHn*: EEG electrode number**

where $l$ is the frequency start point and $l + N - 1$ is the frequency endpoint of the specific frequency band (ie, theta, alpha, beta or gamma). As an example, $ERSP_{CH1-T1}[3]$ means 'third frequency point ERSP value of electrode channel 1 and time point 1' and $f_{CH1-T1}$ means 'averaged ERSP value of electrode channel 1 and time point 1'. The number of total ***Tm*** is 11 for each single time window. These averaged ERSP values from each electrode channel and time point were concatenated to form the time varying EEG feature vector:

$$F_{Odor-frequency\ band-T-P} = [f_{CH1-T1}[l], f_{CH1-T2}[l], f_{CH1-T3}[l] \cdots \cdots, f_{CHn-Tm}[l]]_P \quad (2)$$

***Odor*: Odor condition (i.e., HA, AP, TP)**

***frequency band*: EEG frequency band (i.e., theta, alpha, beta, gamma)**

***T*: Time *window* (i.e., 0-100 ms, 100-200 ms, 200-300 ms, 300-400 ms, 400-500 ms)**

***P*: Participant**

where $F_{Odor-frequency\ band-T-P}$ signifies 'extracted ERSP data of each frequency band, time window, and odor condition from each participant'.

For the dimensional reduction analysis,

$$f_{CHn-Tm}[l] = \frac{1}{P}\sum_{1}^{P}\frac{1}{N}\sum_{l}^{l+N-1} ERSP_{CHn-Tm}[l] \quad (3)$$

***Tm:** ERSP time point*

***CHn:** EEG electrode number*

***P:** Participant number*

where $l$ is the frequency start point and $l + N - 1$ is the frequency endpoint of the specific frequency

band (ie, theta, alpha, beta, or gamma) and arrange the input matrix as,

$$\boldsymbol{F_{Odor-frequency\ band-CH}} = [\boldsymbol{f_{T1}[l]}, \boldsymbol{f_{T2}[l]}, \boldsymbol{f_{T3}[l]} \cdots\cdots, \boldsymbol{f_{Tm}[l]}]_{CH} \quad (4)$$

***Odor:*** *Odor condition (i.e., HA, AP, TP)*

***frequency band:*** *EEG frequency band (i.e., theta, alpha, beta, gamma)*

***T:*** *Time window (i.e., 0-50 ms, 50-100 ms, 100-150 ms, 150-200 ms, 200-250 ms, 250-300 ms, 300-350 ms, 350-400 ms)*

***P:*** *Participant*

where $F_{Odor-frequency\ band-CH}$ signifies 'extracted ERSP data of each frequency band, time window and odor condition from each CH'

### 4.6 Classification design and procedure

Following EEG data extraction, 576 vectors were extracted (3 odor X 1 frequency band X 24 participant X 8 time window) from ERSP. Each vector represents the spatiotemporal activity from the EEG which contains odor information. Because I focused on how odors induce similar brain activities in view of specific frequency bands and time points, a single data set is $\boldsymbol{F_{Odor-frequency\ band-T-P}}$ while $\boldsymbol{frequency\ band}$ and $\boldsymbol{T}$ are fixed.

Using these extracted data sets, LIBSVM (Library for Support Vector Machines, https://www.csie.ntu.edu.tw/~cjlin/libsvm/) was used to decode odor information and quantify similarity between odors. I trained the SVM classifier to separate pairs of odor as different classes (Figure 26-A, middle panel), and then tested the other odor to verify how this odor was classified between two classes (Figure 26-A, right panel). Because different odor sets were used in training and testing, significance could be achieved only when the ERSP patterns reflect similarities among odor qualities. Each time window was separately trained and tested. Next, odor similarity verification was performed (Figure 26-B). Briefly, these odors were defined as inducing similar brain activities when two tested odors were

classified as within a similar class by the SVM classifier. Other cases may represent less or more similarity between odors.

## 5 Statistics and analysis

Results are shown as mean ± SEM and the significance threshold was set at $p<0.05$ ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p<0.001$). To verify survey rates of odor response, Friedman's ANOVA was performed. To verify column differences, I used the Bonferroni post-hoc test. Principal Component Analysis (PCA) was used for a dimensionality reduction method. For clustering analysis, I used density-based spatial clustering of applications with noise (DBSCAN).

# V. Verifying performance of olfactory EEG signal within 200ms

## 1 Background

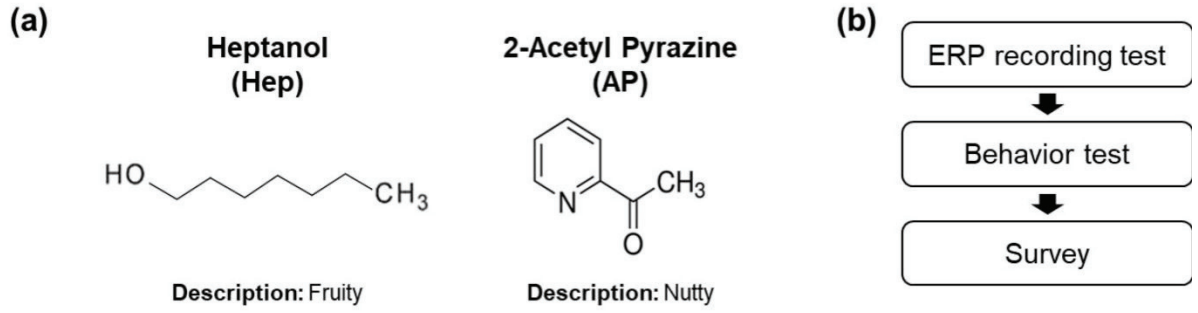
I first focused on observing direct signals from olfactory processing at early time points during olfaction, as primary olfactory areas start to activate within 200 ms. Although EEG has high performance of temporal resolution, olfactory processing occurs in deep brain structures. There are several possibilities that suggest EEG was capable of measuring the olfactory signal. In prior ERP studies investigating the auditory field, they measured ERP signal which originated from the brainstem [85]. However, most EEG studies focused on periods over 200 ms. In recent studies, ERP signals with negative potential at 200–700 ms (N1) and positive potential at 300–800 ms (P2) changed during odor habituation [27,40].

Some lines of evidence suggest that the time of odor signal processing in the human brain could be earlier than 200 ms, similar to findings from animal studies. Previous odor habituation EEG studies in humans [27,40,52,106] have focused mainly on odor signals in the brain after 200 ms, based in part on previous studies of OSNs from the olfactory epithelium [39,95]. These studies report that the time of odor signal processing by OSNs was approximately 300 ms. However, they did not consider factors affecting processing time such as airflow, mucus, and odorant receptor family. Moreover, there was a discrepancy between the time ranges of ERP signals in these studies and the time of odor signal processing in the primary (e.g. PC) and secondary olfactory cortex (e.g. OFC). Recent studies based on electroencephalograms (EEG) and Magnetoencephalography (MEG) indicate that the olfactory signal could reach the PC in approximately 40 ms [87,117]. Furthermore, direct neuronal electrical signal data from human epileptic brains suggest that the PC and OFC process odor information earlier than 200 ms [56]. Behavioral evidence indicates that respiration can be controlled in response to odors within 160 ms, and odor discrimination could happen within 400 ms [68]. These lines of evidence suggest that the odor signal is processed in the brain earlier than 200 ms.

To further verify that EEG originated from odor signaling, I used a habituation condition. Several studies

have shown that the activity of olfactory-related brain areas change with odor habituation conditions. In previous animal studies, both mice and monkeys had decreases in electrical and blood-oxygen-level-dependent (BOLD) signals in the PC were during odor habituation [8,9,60,79,128,138]. Furthermore, these studies showed that modulating neurons in the PC that express glutamate receptors affects odor habituation behavior [8,9,138]. Under odor habituation conditions in humans, BOLD signals decrease in the PC and increase in the OFC [96,98]. Early ERP components are also related to habituation condition. In the case of N1 (appears ~100 ms) and P2 components (appears ~200 ms) in other sensory systems, exogenous stimuli decreased under habituation conditions [14,88,110,125]. These studies suggest that it may be a direct signal when that changes early in ERP signals during olfactory processing.

The purpose of the current study was to find whether the olfactory ERP occurring within 200 ms of odors changes during the odor signal process in the brain when the odors were habituated. If direct odor signal processing in the brain occurring within 200 ms was captured by EEG signals, the olfactory ERP of odors should reflect a change when odors were habituated. If it is not, the olfactory ERP of odors within 200 ms would not differ. I conducted a behavioral test and ERP measurements during odor habituation in human participants. Previous studies have shown that continuous exposure to the same odors during a 30 s period will induce changes in the brain corresponding to odor signals and behavioral responses [8,128]. Thus, in the current experiment, odors were offered during the entire 30 s time-period to induce odor habituation (Figure 15). Data on behavioral responses and brain signals induced by the offered odors that occurred immediately after the 30 s time-period were collected. The amplitude and latency of olfactory ERPs were analyzed at 40–200 ms to examine changes in signaling during odor habituation. The maximum peaks of negative potential (NP) and positive potential (PP) at 40–200 ms were chosen because 40 ms is the earliest time point when the electrical signals induced by odors can be detected in the human brain, whereas 200 ms is the time point when behavioral responses may have already occurred depending on the odor intensity and signaling in the olfactory cortex [68,87,117]. I also examined the correlation between the results of behavioral tests and ERP data within 200 ms to confirm that ERP data was related to the behavioral test.




(c)

| | Step 1 | Step 2 | Step 3 |
|---|---|---|---|
| | Habituation 30s | Test | Rest period 30s |
| None | Distilled water | Hep | |
| | | AP | |
| Different | AP | Hep | |
| | Hep | AP | |
| Same | Hep | Hep | |
| | AP | AP | |

**Figure 15. Experimental design**. a. Heptanol and 2-acetyl pyrazine were used in this experiment. These odorant compounds have different chemical structures and descriptions (The Good Scents Company Information System). b. Overview of tests used in this study c. ERP recording test and behavioral test consisted of three steps. The first step was habituation. To induce habituation, odors or a distilled water control sample were offered continuously during a 30 s period. The second step was the test where one of two odors was offered to measure the intensity of the corresponding brain signals. The last step was a 30 s rest period before the next round of the experiment. There were three different habituation conditions. “None”: distilled water was offered in the first step and one of the two odors in the second step. “Different”: if 2-acetyl pyrazine was offered in the first step, heptanol was offered in the second step, and vice versa. “Same”: the same odorant compound was offered in the first and second steps.

## 2 Results

### 2.1 ERP signal observed during olfaction before 200 ms

I first established that ERP signals can be measured before 200 ms in odor condition. For characterizing ERP signals within 200 ms, I calculated the SNR of peaks of the NP or PP from 40 ms to 200 ms. I found that the SNR of the NP (1.368 ±0.0366) and PP (1.343 ±0.0367) was statistically higher than the noise level (Table 3). I examined the SNR of the NP and PP across 64 channels as well (Table 4 and Table 5) and found results consistent with those in Table 3, indicating that over 30 channels were statistically higher than the noise level in both the NP and PP.

**Table 3. Significantly higher values in the signal to noise ratio (SNR) of ERP 40 ms – 200 ms.** In total, 64 channels were used. The SNR of NP and PP was significantly higher than the noise level in the one sample t-test from 40 ms to 200 ms across all participants.

| SNR of ERP 40ms - 200ms | | | | | | | |
|---|---|---|---|---|---|---|---|
| Negative Potential (NP) | | | | Positive Potential (PP) | | | |
| **Mean** | **Df** | **SEM** | **P value** | **Mean** | **Df** | **SEM** | **P value** |
| 1.368 | 12 | ±0.0366 | P < 0.0001 | 1.343 | 12 | ±0.0367 | P < 0.0001 |

**Table 4. One sample T-test of the SNR of the NP across 64 channels.** The SNR of the NP across 64 channels was analyzed by one sample t-tests comparing noise levels (value 1).

| SNR of Negative potential | | | | | |
|---|---|---|---|---|---|
| **CH** | **Mean** | **P value** | **CH** | **Mean** | **P value** |
| **Fp1** | 1.17 | 0.254 | **FPz** | 1.12 | 0.591 |
| **AF7** | 1.10 | 0.630 | **FP2** | 1.21 | 0.310 |
| **AF3** | 1.29 | 0.243 | **AF8** | 1.01 | 0.922 |
| **F1** | 1.06 | 0.779 | **AF4** | 1.22 | 0.180 |
| **F3** | 1.47 | 0.024 | **AFz** | 1.26 | 0.221 |
| **F5** | 1.10 | 0.645 | **Fz** | 1.17 | 0.395 |
| **F7** | 1.09 | 0.601 | **F2** | 1.15 | 0.507 |
| **FT7** | 1.40 | 0.021 | **F4** | 0.87 | 0.431 |
| **FC5** | 0.96 | 0.773 | **F6** | 0.91 | 0.560 |
| **FC3** | 1.39 | 0.029 | **F8** | 1.14 | 0.407 |
| **FC1** | 1.02 | 0.913 | **FT8** | 1.37 | 0.079 |
| **C1** | 1.37 | 0.056 | **FC6** | 0.97 | 0.883 |
| **C3** | 1.63 | <0.01 | **FC4** | 1.14 | 0.470 |
| **C5** | 1.26 | 0.016 | **FC2** | 1.10 | 0.563 |
| **T7** | 1.47 | <0.01 | **FCz** | 1.13 | 0.488 |
| **TP7** | 1.64 | <0.01 | **Cz** | 1.27 | 0.121 |
| **CP5** | 1.56 | <0.01 | **C2** | 1.16 | 0.359 |
| **CP3** | 1.40 | 0.027 | **C4** | 1.45 | 0.033 |
| **CP1** | 1.52 | <0.01 | **C6** | 1.41 | 0.075 |
| **P1** | 1.82 | <0.01 | **T8** | 1.18 | 0.344 |
| **P3** | 1.49 | <0.01 | **TP8** | 1.64 | <0.01 |
| **P5** | 1.56 | <0.01 | **CP6** | 1.56 | 0.015 |
| **P7** | 1.65 | <0.01 | **CP4** | 1.51 | <0.01 |
| **P9** | 1.58 | <0.01 | **CP2** | 1.42 | 0.042 |
| **PO7** | 1.49 | <0.01 | **P2** | 1.62 | <0.01 |
| **PO3** | 1.60 | <0.01 | **P4** | 1.60 | <0.01 |
| **O1** | 1.63 | <0.01 | **P6** | 1.52 | 0.024 |
| **Iz** | 1.56 | <0.01 | **P8** | 1.63 | <0.01 |
| **Oz** | 1.58 | <0.01 | **P10** | 1.68 | <0.01 |
| **POz** | 1.62 | <0.01 | **PO8** | 1.70 | <0.01 |
| **Pz** | 1.55 | <0.01 | **PO4** | 1.54 | <0.01 |
| **CPz** | 1.26 | 0.186 | **O2** | 1.60 | <0.01 |

**Table 5. One sample T-test of the SNR of the PP across 64 channels.** The SNR of the PP across 64 channels was analyzed by one sample t-tests comparing noise levels (value 1).

| SNR of Positive potential | | | | | |
|---|---|---|---|---|---|
| **CH** | **Mean** | **P value (PP)** | **CH** | **Mean** | **P value (PP)** |
| **Fp1** | 1.34 | 0.083 | **FPz** | 1.30 | 0.143 |
| **AF7** | 1.22 | 0.230 | **FP2** | 1.22 | 0.141 |
| **AF3** | 1.32 | 0.057 | **AF8** | 1.40 | 0.012 |
| **F1** | 1.58 | <0.01 | **AF4** | 1.61 | <0.01 |
| **F3** | 1.62 | <0.01 | **AFz** | 1.50 | <0.01 |
| **F5** | 1.42 | 0.044 | **Fz** | 1.61 | <0.01 |
| **F7** | 1.36 | 0.083 | **F2** | 1.53 | <0.01 |
| **FT7** | 1.10 | 0.630 | **F4** | 1.64 | <0.01 |
| **FC5** | 1.44 | 0.028 | **F6** | 1.56 | <0.01 |
| **FC3** | 1.47 | 0.031 | **F8** | 1.54 | <0.01 |
| **FC1** | 1.67 | <0.01 | **FT8** | 1.34 | 0.040 |
| **C1** | 1.51 | <0.01 | **FC6** | 1.47 | <0.01 |
| **C3** | 1.19 | 0.350 | **FC4** | 1.48 | <0.01 |
| **C5** | 1.45 | 0.025 | **FC2** | 1.57 | <0.01 |
| **T7** | 1.43 | 0.037 | **FCz** | 1.43 | <0.01 |
| **TP7** | 1.03 | 0.897 | **Cz** | 1.47 | 0.022 |
| **CP5** | 1.32 | 0.099 | **C2** | 1.55 | <0.01 |
| **CP3** | 1.41 | 0.033 | **C4** | 1.38 | <0.01 |
| **CP1** | 1.32 | 0.082 | **C6** | 1.40 | 0.034 |
| **P1** | 0.95 | 0.745 | **T8** | 1.58 | <0.01 |
| **P3** | 1.18 | 0.238 | **TP8** | 1.17 | 0.430 |
| **P5** | 0.86 | 0.394 | **CP6** | 1.27 | 0.209 |
| **P7** | 1.04 | 0.816 | **CP4** | 1.44 | 0.020 |
| **P9** | 1.19 | 0.339 | **CP2** | 1.52 | <0.01 |
| **PO7** | 1.21 | 0.298 | **P2** | 1.31 | 0.058 |
| **PO3** | 1.05 | 0.778 | **P4** | 1.15 | 0.435 |
| **O1** | 1.20 | 0.317 | **P6** | 1.32 | 0.136 |
| **Iz** | 1.28 | 0.182 | **P8** | 1.05 | 0.800 |
| **Oz** | 1.47 | <0.01 | **P10** | 1.26 | 0.127 |
| **POz** | 1.16 | 0.393 | **PO8** | 1.12 | 0.445 |
| **Pz** | 1.14 | 0.417 | **PO4** | 1.07 | 0.760 |
| **CPz** | 1.39 | 0.028 | **O2** | 1.40 | 0.040 |

### 2.2 The perceived intensity of the odor decreased when the same odor was offered again

To verify whether odor habituation occurred under the “same” condition, the perceived intensities of the odors offered in the behavioral test were compared across the conditions (Figure 16). Intensity scores were significantly different across the conditions ($F[2|38]=37.56$, $p<0.0001$, $\varepsilon=0.81$, $p\eta^2=0.76$; RMANOVA). The power value for 13 sample sizes in the behavioral test was 1 (significance level 0.05). The intensity score was significantly lower in the “same” condition than in the “none” condition ($T=8.13$, $p<0.0001$; Bonferroni’s test) and “different” condition ($T=5.70$, $p<0.0001$; Bonferroni’s test). Pleasantness ($T[12]=0.71$, $p=0.49$) and intensity ($T[12]=0.28$, $p=0.78$) scores of heptanol were not significantly different from those of 2-acetyl pyrazine (Figure 17).

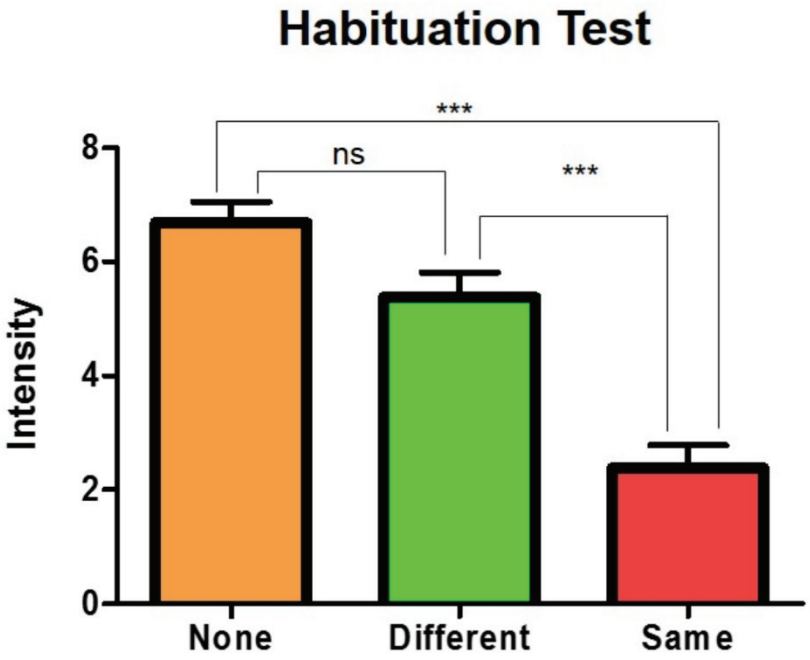



**Figure 16. Decreases in odor intensity when the same odor was offered**. The intensities of the odors offered in the test step were compared across the three conditions. Odor intensity was significantly lower under the “same” condition than under “none” and “different” conditions, but did not differ significantly between “none” and “different” conditions. ***$p$<0.001

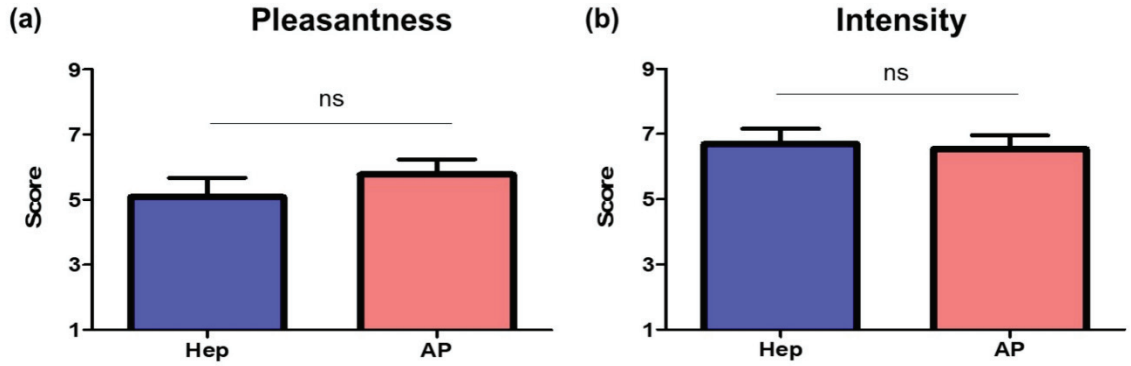



**Figure 17. No significant differences in intensity or pleasantness of two odors.** The y-axes show the pleasantness and intensity scores of the two odors evaluated using a 9-point Likert scale questionnaire. Pleasantness and intensity scores of heptanol (Hep) were not significantly different from those of 2-acetyl pyrazine (AP).

### 2.3 Significant changes in amplitude and latency of negative and positive potentials within 200 ms

To examine changes in ERP amplitude, I examined differences in the NP across the conditions. I found that four channels showed significant differences (Table 6a). Specifically, three channels showed significantly different amplitudes across the conditions: C2, C6, and F5 channels (Table 6a, left panel; C2 channel: $F[2|38]=3.97$, $p\eta^2=0.25$; C6 channel: $F[2|38]=3.49$, $p\eta^2=0.23$; F5 channel: $F[2|38] = 3.79$, $p\eta^2=0.24$; $p<0.05$, $\varepsilon>0.75$ each, RMANOVA). In C2 and C6, the NP amplitude tended to be lower under the "same" condition than under the other two conditions. In the C2 channel, the NP amplitude was significantly lower under the "same" condition than under the "none" condition ($T=2.81$, $p<0.05$; Bonferroni's test). On the other hand, in the F5 channel, the NP amplitude tended to be lower under the "none" than under the other two conditions. The CP6 channel showed significantly different latencies across the conditions (Table 6a, right panel; $F[2|38]=3.76$, $p=0.038$, $\varepsilon=0.72$, $p\eta^2=0.24$; RMANOVA). In the CP6 channel, the NP latency under the "none" condition was significantly later than the latency under the "different" condition ($T=2.72$, $p<0.05$; Bonferroni's test) and tended to be later under the "none" condition than under the "same" condition.

Thirteen channels showed significant differences (Table 6b). Specifically, eight channels showed significantly different PP amplitudes across the conditions: C4, CP6, FC5, CP3, CP1, P1, P3 and Pz channels (Table 6b, left panel; C4: $F[2|38]=3.65$, $p\eta2=0.23$; CP6: $F[2|38]=4.58$, $p\eta2=0.28$; FC5: $F[2|38]=3.71$, $p\eta2=0.24$; CP3: $F[2|38]=3.95$, $p\eta2=0.25$; CP1: $F[2|38]=5.59$, $p\eta2=0.32$; P1: $F[2|38]=6.16$, $p\eta2=0.34$; P3: $F[2|38]=3.52$, $p\eta2=0.23$; Pz: $F[2|38]=6.29$, $p\eta2=0.34$; $p<0.05$, $\varepsilon>0.75$ each, RMANOVA). Among these eight channels, FC5 and CP3 showed a tendency for the PP amplitude to be lower under the "same" condition than under the two other two conditions. Bonferroni's post-test suggested that FC5 ($T=2.66$, $p<0.05$) and CP3 ($T=2.81$, $p<0.05$) showed significant differences in the amplitudes of between the "none" and "same" conditions. The C4 and CP6 channels showed a tendency for the PP amplitude to be higher under the "same" condition than under the other two conditions. Bonferroni's post-test found that CP6 ($T=2.97$, $p<0.05$) showed significant differences in the amplitudes of between the "none" and "same" conditions. The rest of the channels showed a different pattern: they had the highest amplitude under the "different" condition. In the CP1, P1, and Pz channels, the PP amplitude was significantly higher under the "different" condition than the "none" condition (CP1: $T=3.28$, $p<0.01$; P1:

T=3.50, p<0.01; Pz: T=3.53, p<0.01; Bonferroni's test). Five channels had significant differences in PP latency across conditions: AF8, C3, C5, Cz and CPz channels (Table 6b, right panel; AF8: F[2|38]=3.74, pη2=0.24; C3: F[2|38]=3.71, pη2=0.24; C5: F[2|38]=3.50, pη2=0.23; Cz: F[2|38]=4.27, pη2=0.26; CPz: F[2|38]=7.69, pη2=0.39; p<0.05, ε>0.75 each, RMANOVA). In C3, Cz, and CPz, latency tended to be slower under the "same" condition than under the other two conditions. In the CPz channel, the PP latency was significantly slower under the "same" condition than under the "different" condition (T=3.33, p<0.01; Bonferroni's test) and "none" condition (T=3.46, p<0.01; Bonferroni's test). In the AF8 channel, the PP latency was slower under the "different" condition than under the other two conditions; in the C5 channel, the PP latency was slower under the "none" condition than under the other two conditions. In the post-test, the PP latency in the AF8 channel was significantly slower under the "different" condition than under the "none" condition (T=2.64, p<0.05; Bonferroni's test).

These results suggest that the NP and PP within 200 ms changed during odor habituation. In the case of the NP, the amplitude under the "same" condition changed in two channels (C2 and C6) compared to that under the other two conditions. In the case of PP, the amplitude under the "same" condition changed in four channels (FC5, CP3, C4, and CP6), and the latency under the "same" condition changed in three channels (C3, Cz, and CPz).

**Table 6. Channels with significant differences across the conditions in the amplitude and latency of NP and PP (40–200 ms).**

**(a)**

| ERP component | Channel | Amplitude (μV) | | | | Latency (ms) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | None | Different | Same | F-value | None | Different | Same | F-value |
| Negative potential (40–200 ms) | Right hemisphere | | | | | | | | |
| | C2 | –1.38 | –0.93 | –0.59 | 3.97* | 131 | 125 | 100 | 1.11 (ns) |
| | C6 | –1.29 | –1.26 | –0.57 | 3.49* | 143 | 114 | 126 | 1.36 (ns) |
| | CP6 | –1.41 | –1.25 | –1.18 | 0.62 (ns) | 146 | 100 | 128 | 3.76* |
| | Left hemisphere | | | | | | | | |
| | F5 | –1.51 | –2.68 | –2.60 | 3.79* | 97 | 106 | 125 | 0.87 (ns) |

**(b)**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Positive potential (40–200 ms) | Right hemisphere | | | | | | | | |
| | AF8 | 2.80 | 2.65 | 2.46 | 0.14 (ns) | 102 | 156 | 116 | 3.73* |
| | C4 | 1.47 | 1.47 | 2.06 | 3.65* | 105 | 121 | 130 | 0.57 (ns) |
| | CP6 | 1.11 | 1.38 | 1.94 | 4.62* | 119 | 128 | 120 | 0.09 (ns) |
| | Left hemisphere | | | | | | | | |
| | FC5 | 1.71 | 1.12 | 0.85 | 3.71* | 136 | 117 | 119 | 0.41 (ns) |
| | C3 | 0.98 | 1.27 | 1.18 | 0.47 (ns) | 134 | 87 | 141 | 3.71* |
| | C5 | 1.12 | 1.02 | 0.79 | 1.21 (ns) | 148 | 112 | 100 | 3.50* |
| | CP3 | 1.43 | 1.78 | 1.02 | 3.95* | 131 | 118 | 152 | 1.32 (ns) |
| | CP1 | 1.20 | 1.96 | 1.45 | 5.59* | 97 | 102 | 136 | 2.21 (ns) |
| | P1 | 1.23 | 2.45 | 1.90 | 6.16** | 110 | 109 | 108 | 0.01 (ns) |
| | P3 | 1.77 | 2.46 | 1.93 | 3.52* | 128 | 132 | 130 | 0.02 (ns) |
| | Central position | | | | | | | | |
| | Cz | 1.91 | 2.30 | 1.90 | 0.57 (ns) | 124 | 106 | 152 | 4.27* |
| | Pz | 1.43 | 2.87 | 2.02 | 6.29** | 111 | 112 | 128 | 0.47 (ns) |
| | CPz | 1.56 | 2.34 | 1.84 | 2.59 (ns) | 107 | 108 | 160 | 7.68** |

In total, 64 channels were used. a. NP. Four channels showed significant differences in NP amplitudes or latency. C2 and C6 showed significantly higher NP amplitude under the “none” or “different” condition than under the “same” condition in the right hemisphere. F5 showed significantly higher NP amplitude

under “different” or “same” than under the “none” condition in the left hemisphere. CP6 showed significantly different NP latency across conditions in the right hemisphere. b. PP. Thirteen channels showed significant differences in PP amplitudes or latency. C4 and CP6 showed significantly higher PP amplitude under the “same” condition than under the other two conditions in the right hemisphere. FC5, CP3, CP1, P1, and P3 showed significantly different values across conditions in the left hemisphere. FC5 and CP3 showed higher values under “none” and “different” conditions than under the “same” condition; CP1, P1, and P3 showed higher values under the “different” condition than under the other two conditions. Pz in the central position showed a lower value under the “none” condition than under the other two conditions. AF8, CP6, C3, C5, Cz, and CPz showed significant differences in latency. AF8 and CP6 showed higher values under “different” condition than under the other two conditions in the right hemisphere. C3 showed a lower value under “different” condition, whereas C5 showed a higher value under the “none” condition in comparison with the other two conditions in the left hemisphere. Cz showed a lower value under the “different” condition, and CPz showed a higher value under the “same” condition in comparison with the other two conditions.

### 2.4 Changes in the NP and PP patterns across the conditions are related to the behavioral test

Although my analysis showed that the NP and PP within 200 ms changed during odor habituation, further observations and comparison between the ERP (i.e., NP, PP) and the behavioral test were necessary to understand their relationships. Using a correlational analysis, I compared the relationship between the amplitude and latency of ERPs across the conditions and behavioral results.

In three channels, I found significant positive correlations between the NP amplitude and behavior among whole channels (Figure 18a–d; CP1: r=0.35, p=0.031; C6: r=0.49, p=0.002; FT8: r=0.36, p=0.026), and the C6 channel showed significantly different NP amplitudes across the conditions (Figure 18c). The F7 channel showed a significant negative correlation between NP latency and the results of the behavioral test, but there were no significant differences across the conditions (Figure 18f).

In three channels, I found significant correlations between the PP amplitude and behavior among the whole channels (Figure 19a): the CP3 channel showed a positive correlation (Figure 19b; r=0.36, p=0.023), whereas the C6 and CP6 channels showed negative correlations (Figure 19c, d; C6: r=−0.34, p=0.028; CP6: r=−0.33, p=0.042). Moreover, the CP3 and CP6 channels showed significantly different PP amplitudes across the conditions, and the C6 channel had a similar tendency to that of the CP6 channel, although there was less statistical significance in the C6 channel. Five channels showed a significant correlation between PP latency and the results of the behavioral test (Figure 19e). Specifically, C1, CPz, F5, FC2, and Cz channels showed a negative correlation (Figure 19f–j, C1: r=−0.34, p=0.036; CPz: r=−0.35, p=0.028; F5: r=−0.34, p=0.034; FC2: r=−0. 34, p=0.032; Cz: r=−0.34 p=0.032). In the CPz and Cz channels, I also found a significantly different PP latency across the conditions (Figure 19g, j).

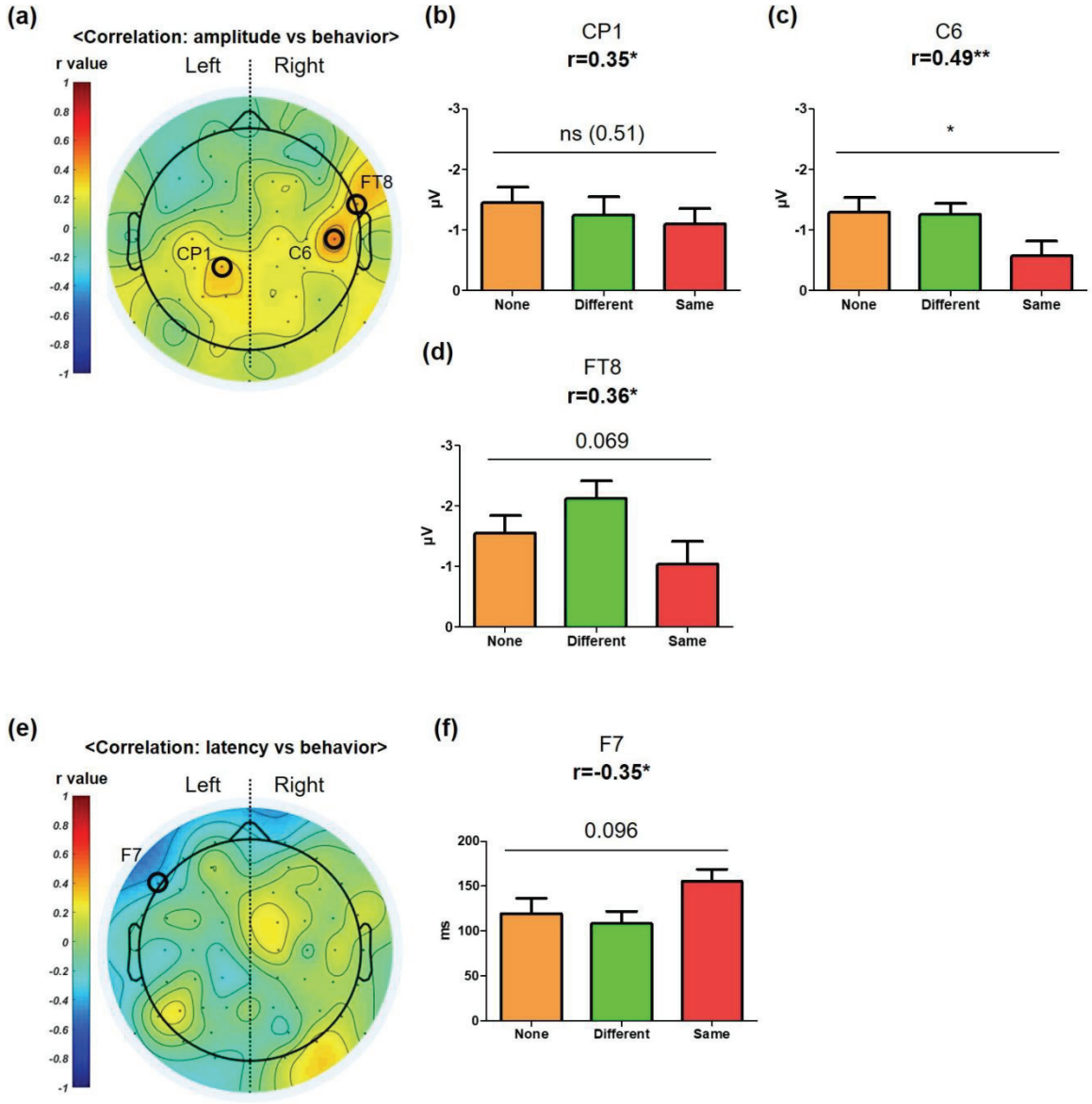



**Figure 18. Correlations between the NP and behavior (40–200 ms NP vs. behavioral results).** Topographical patterns of correlation between NP and behavior. Circled channels showed statistically significant correlations. a. Topographical patterns of correlation between the NP amplitude and the behavior. Three channels showed significant correlations (CP1, C6, and FT8). b–c. CP1, C6, and FT8 significantly correlated with behavior (r-value ≥ 0.35). In the RMANOVA, C6 also showed a statistically significant decrease, and FT8 showed a tendency for a decrease under the "same" condition. e. Topographical patterns of correlation between NP latency and behavior. F7 showed a significant negative correlation with behavior (r-value = −0.35). F7 was not significantly different in NP latency across conditions. *$p < 0.05$, **$p < 0.01$

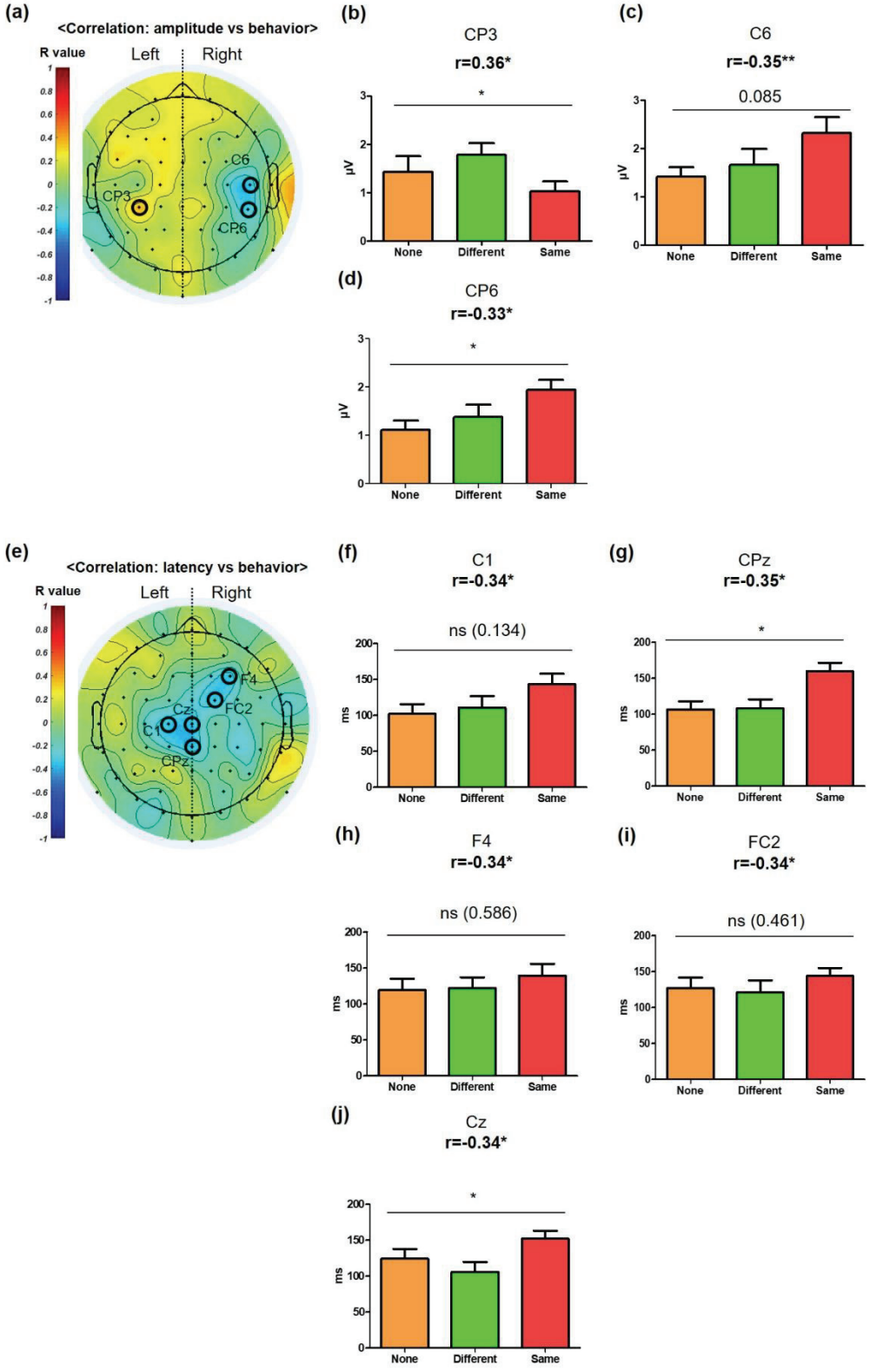



**Figure 19. Correlations between the PP and behavior (40–200 ms PP vs. behavioral results).** Topographical patterns of correlation between PP and behavior. Circled channels showed a statistically significant correlation. a. Topographical patterns of correlation between the PP amplitude and behavior. Three channels showed significant correlations (CP3, C6, and CP6). b–c. CP3, C6, and CP6 significantly correlated with behavior (|*r* value| ≥ 0.33). In the RMANOVA CP3 showed a statistically

significant decrease, and CP6 showed a statistically significant increase under the “same” condition, whereas C6 showed a tendency for an increase under this condition. e. Topographical patterns of correlation between PP latency and behavior. Five channels showed significant correlations with behavior (C1, CPz, F4, FC2, Cz). f–j. C1, CPz, F4, FC2, and Cz significantly correlated with behavior (r-value ≥ 0.34). In the ANOVA, CPz showed a significant decrease, and Cz showed a significant increase under the “same” condition. C1, F4, and FC2 showed a tendency for an increase under the “same” condition. *$p < 0.05$, **$p < 0.01$

## 3 Discussion

I found that the direct odor signal can be captured by EEG measurement within 200 ms. Specifically, ERP signals detected in the odor stimulation conditions, and ERP signals of odors within 200 ms differed when the odors were habituated, suggesting that these changes were related to odor habituation. I firstly found that ERP signal observed during odor conditions within 200ms (Table 3). To further confirm these results, I designed an experimental procedure to test odor habituation (Figure 15). I offered odors to participants for 30 s for desensitization of OSNs in the OE. For the behavioral test, I chose heptanol and 2-acetylpyrazine, which are perceptually and structurally different odors, and confirmed that the intensity of odor was significantly decreased under the “same” condition, not but under “different” and “none” conditions (Figure 16). These results suggest that odor habituation occurred mainly when the same odors were repeated, whereas cross adaptation caused by the two odors was barely detectable. This result is in line with previous odor habituation studies [96,97]. In the experimental setting, I found that ERPs differed within 200 ms depending on the conditions (Table 4 and Table 5). In several channels, the amplitude and latency of the NP or PP were changed within 200 ms under the “same” condition compared with other conditions. To examine whether these ERP changes were related to the behavior, I performed a correlational analysis comparing the ERPs and behavioral results. I found a significant correlation between the ERPs within 200 ms and behavior, mainly in channels located in the right temporal and parietal lobe areas (Figure 18 and Figure 19), implying that information for odor habituation may be processed centrally at early time points.

Results suggest that ERP signals in the human brain can be modulated at very early time points (within 200 ms) by odor habituation as animal studies suggest. This evidence implies the involvement of the primary and secondary olfactory cortex. Previous studies regarded the involvement of the central nervous system as a major mechanism of odor habituation [8,9,79,96,138]. Not only did neuronal activities in the PC and OFC change during odor habituation, but modulating neuronal activities in PC can similarly modify odor habituation behavior [9,98,128,138]. Findings suggest that the olfactory cortex is involved in odor habituation. Within 200 ms, the PC and OFC are activated mainly by odors [87,117] and odor-specific information may be processed in the PC [56]. If the olfactory cortex processes odor signals within 200 ms and is involved in odor habituation, brain activity may change within 200 ms during odor habituation.

The current data show that olfactory ERP signals within 200 ms change during odor habituation, suggesting that the olfactory cortex may modulate odor processing at very early time points during odor habituation.

Additionally, I found that the odor signal during odor habituation is asymmetrically processed in the brain. In the current study, the NP amplitude within 200 ms showed a positive correlation with behavior mainly in the right hemisphere (Figure 18a). On the other hand, the PP amplitude within 200 ms showed a negative correlation with behavior mainly in the right hemisphere (Figure 19a). These data are in line with the pattern of EEG topographical data at 155 ms in a previous study [87], which reported activation of the secondary olfactory cortex in the inferior frontal OFC, left superior OFC, and left gyrus rectus. The asymmetric pattern of topographical data is also supported by findings from a previous fMRI study on odor habituation in human subjects, which found that the decrease in the activity of the PC was not the same bilaterally [98].

The current ERP data showed smaller SNR than previous olfactory ERP studies [10,40]. Although this could be an issue in ERP interpretation which should be based on the actual signals but not on the noise signals, my results were based on the ERP data that indicated statistically higher signals compared to the noise level (Table 3). This SRN result suggests that my results were based on actual signals rather than noise signals. One possible explanation for the smaller SNR of my data might be due to the time period (40 ms – 200 ms) of my ERP data. Within 200 ms, the primary olfactory cortex and OFC are mainly activated by odors [87,117]. These brain areas are located deep in the brain (Brodmann area 11, 27) and activities of these areas might be the main sources of my ERP signals.

In conclusion, direct odor signals can be captured by EEG within 200 ms. I found that the NP and PP within 200 ms changed in relation to odor habituation, and these results suggest that changing odor conditions can alter the EEG signal within 200 ms. These findings can serve as the basis for analyzing EEG signals for odor categorization within 200 ms which will be presented at a later time.

# VI. Screening of odor categorization features in the brain based on temporal view

## 1 Background

Based on the previous study (study 1), I examined EEG signals during olfaction. As highlighted in the theoretical background, odor identification by the brain occurs at the time and spatial scale of specific neuronal activity [102,114,115,121]. Temporal distribution of odorant-evoked activity in the piriform cortex (PC) was observed in rodents [102]. Additionally, previous research demonstrates that spatial neuronal activity in the PC encodes odor information [61,107,134]. Increasing evidence from rodent studies confirms the importance of the orbitofrontal cortex (OFC) in mediating odor information processing [107,108]. Subsequent evidence suggests that populations of neurons coding activity of the PC may be key in odor processing [89] and specific frequencies (e.g., beta and gamma) may be related to olfactory functions [86]. These studies provide precise information related to odor discrimination. Indeed, some studies characterizing temporal activities during odor processing suggest that olfactory-related areas are activated at approximately 80 ms [117] and olfactory specific activation of the PC starts at 110 ms [56]. There is a lack of understanding of when and how odor categorization occurs during olfactory processing.

For these reasons, I focused on how the brain categorized odor similarities during the olfactory processing period. I used two odors (Figure 20, i.e., 2-acetylpyrazine [AP] and 2, 3, 5-trimethly pyrazine [TP]) that are described as similar by humans. Hexan-1-al (HA) was used as a control odor (i.e., considered distinct from AP and TP). To measure direct brain signals [15] at the time resolution of interest, electroencephalography (EEG) was used. I focused on the 0-400 ms time frame as behavioral evidence suggests that odor discrimination occurs within 420 ms of odor stimulation [72]. EEG frequency signals can provide spatial and temporal insights; EEG is also suitable due to increasing evidence suggesting that neuronal oscillations mediate overall neuronal computations [42,94,109]. Because spatial and temporal neural representations play key roles in the olfactory network [74,115,126], and comparatively more evidence

in rodent studies rather than human event-related potential (ERP) studies, I performed event-related spectral perturbation (ERSP) in spatial and temporal scales. Although EEG topographies do not reflect precise brain region information for neuronal activity, similar spatial patterns of topographies represent similar activation of brain regions. ERSPs are analyzed by theta, alpha, beta, and gamma frequency bands, as each is reportedly indicative of varying olfaction processes. Theta waves have been reported to differ when distinct odors are used for stimulation [70,82] and are further associated with activity in the hippocampus [37,90]. Alpha waves may play a role in odor valence [71], and beta and gamma waves are reportedly critical for odor-information processing in rodents [2,11,22,64,91,124] and humans [6,54].

My primary hypothesis is that brain activity patterns will show little difference for odors perceived as similar, but will differ for odors perceived as distinct. To test this hypothesis, I characterized brain signal patterns when stimulated by various odors.

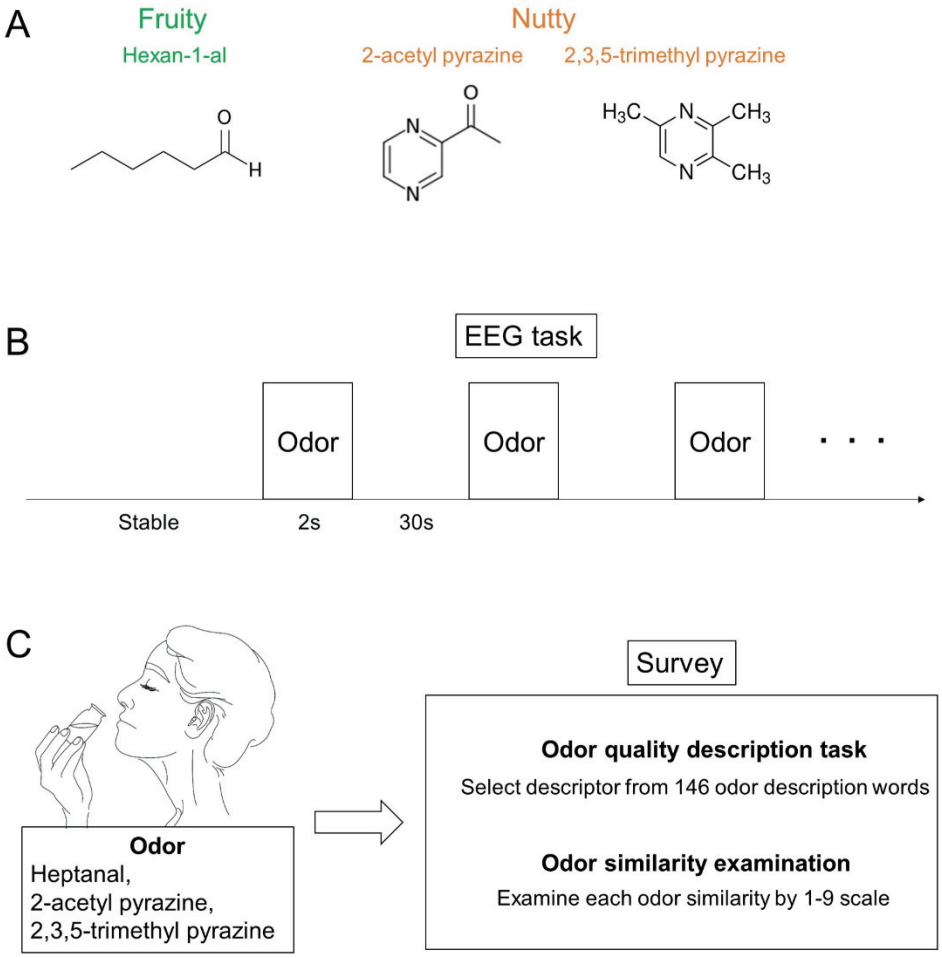



**Figure 20. Experimental design**. A. Odor stimulation. B. EEG experimental procedure (a stable period [60 seconds] was followed by stimulation [2 seconds] and rest [30 seconds]). During the rest period, fixation was performed. C. Survey experimental procedure.

## 2 Results

### 2.1 Odor quality similarity.

Because odor quality can be influenced by intensity and hedonicity, I first confirmed that there were no differences in odor intensity and hedonicity among the odors tested (Figure 21). To verify intensity and hedonicity, participants rated each odor and no significant differences were observed between odors in intensity ($X^2[2,71]=0.67$, $p=0.71$, Friedman's ANOVA) or hedonicity ($X^2[2,71]=2.48$, $p=0.29$, Friedman's ANOVA).

To verify odor similarity, participants were asked to select specific odor descriptors of AP, TP, and HA (Figure 22A). Similar descriptors (e.g., 'nutty', 'roasted', and 'heavy') were chosen for AP and TP, however, those used to describe HA were unique. Second, I conducted a survey to verify similarity between odors (Figure 22B; $X^2[2,71]=32.00$, $p<0.0001$, Friedman's ANOVA). Participants compared two selected odors and rated similarity using a scale of one to ten (larger numbers reflect more similarity). I found that AP and TP were considered more similar than other combinations ($p<0.0001$, Bonferroni post hoc test).

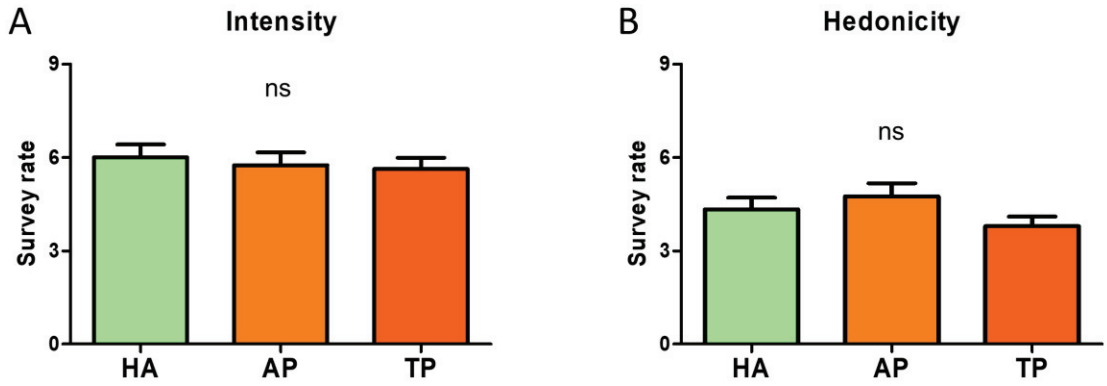



**Figure 21. Intensity and hedonicity between odors.** A-B. Intensity and hedonicity of odors. There were no significant differences between odors.

A

Odor descriptor

| HA | AP | TP |
|---|---|---|
| Apple (20) | | |
| Flower (12) | | |
| Green (23) | | |
| | Almond (19) | |
| | Nutty (24) | Nutty (24) |
| | Roasted (24) | Roasted (24) |
| | Heavy (21) | Heavy (21) |
| | | Pungent (18) |

B

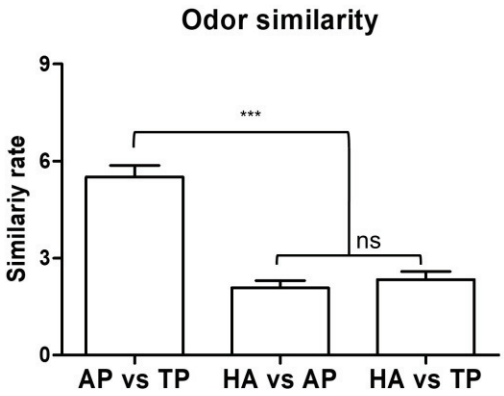



**Figure 22. Similarities between odors**. A. Quality descriptors used for each odor. Presented descriptors are selected by 12 or more participants after odor stimulation. Orange-colored words indicate the same descriptor between AP and TP. C. Similarity survey between odors. Similarity between AP vs TP is significantly higher than AP vs HA and TP vs HA.

### 2.2 AP- and TP-induced spatial patterns of theta ERSP at 0-100 ms and 150-200 ms.

As previous studies suggest that spatial patterns of brain activity can represent odor categorization processes in the brain, I focused on spatial patterns of EEG signals initially. Each data set was divided into eight sets based on time and similarity of spatial patterns between conditions (AP vs TP vs HA) (Figure 23). I found that the theta ERSP reflected similar spatial patterns of AP and TP while remaining distinct from HA. Beta and gamma bands revealed distinct patterns between odor conditions. Specifically, spatial patterns for APs and TPs overlapped at 0-50 ms, 50-100 ms, and 150-200 ms while remaining mostly distinct from HA, though other time windows and frequencies did not show overlapping patterns between AP and TP. To verify these findings, I subsequently performed DBSCAN clustering analysis (Figure 25). I specified minPts for three and epsilon distance for 0.2 (Figure 24). I found that AP and TP clustered in the same class in 0-50 ms, 50-100 ms, and 150-200 ms while HA clustered in different classes.

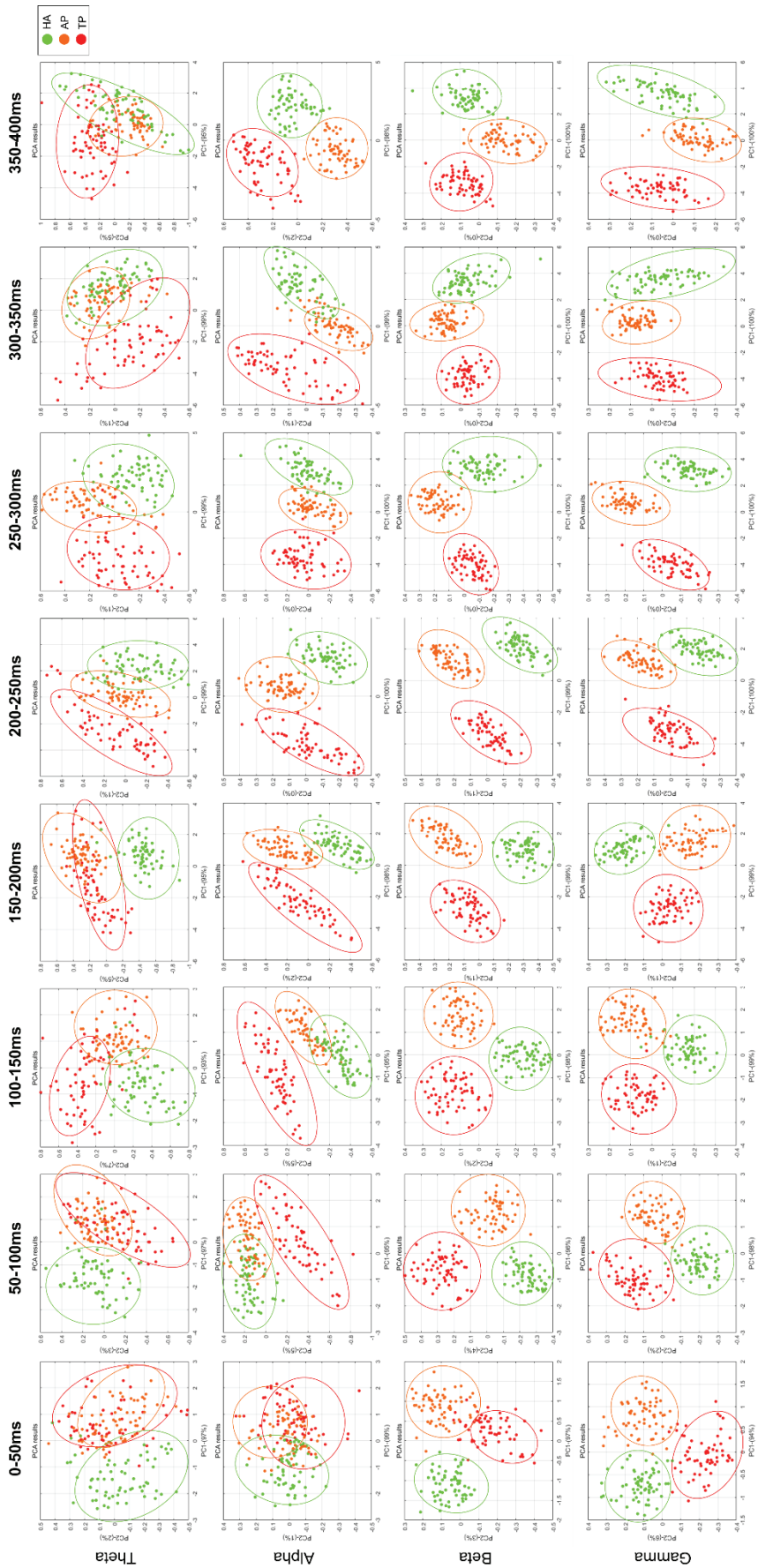



**Figure 23. Dimensionality reduction results of ERSPs from each time window and each frequency band.** Dimensionality reduction results of ERSP data from eight specific time windows and frequency bands. Each dot represents a specific channel. Green dots were HA, orange dots were AP, and red dots were TP

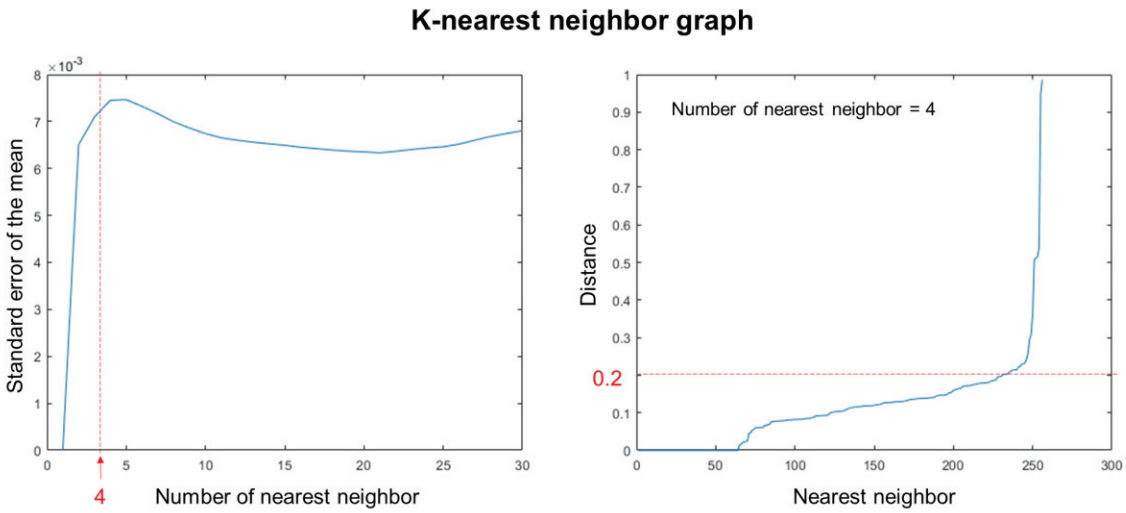



**Figure 24. Specifying minPts and epsilon distance for DBSCAN clustering.** K-nearest neighbor graph from theta PCA data (Figure 23 top row panels). These graphs are representative graphs from AP vs AP of 0-50 ms. Left panel graph is for defining minPts for DBSCAN. From four of the nearest neighbor points, standard error of the mean is shown as a saturated pattern. Because I compared the same data, I specified minPts as three. Right panel graph is for defining epsilon distance for DBSCAN. From a distance point of 0.2, the elbow of the curve was shown. From this data, I specified epsilon distance as 0.2.

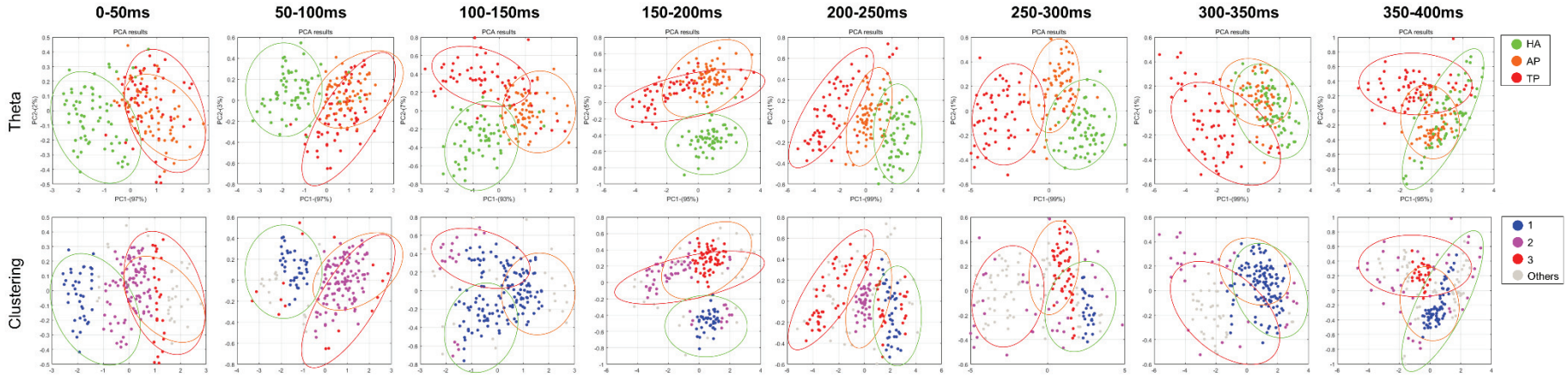



**Figure 25.** AP and TP induce similar ERSP activities in 0-100 ms and 150-200 ms. Clustering results of theta ERSP data from eight specific time window. Each dot represented a specific channel. Top row panels were PCA results of theta and bottom row panels were clustering results by DBSCAN. For top row panels, green dots were HA, orange dots were AP, and red dots were TP. For bottom row panels, each color of dots, except for gray dots, represents a specific cluster. Gray dots were the noise group which consisted of clusters with three or fewer observations.

### 2.3 Multivariate pattern of theta ERSPs indicates that AP and TP induce similar theta ERSPs at 50-100 ms and 150-200 ms.

Although similar spatial patterns of theta ERSPs between AP and TP were found, these results did not consider the variance of participants. To confirm ERSP similarity more precisely, I conducted a multivariate pattern analysis with theta ERSPs (Figure 26A and B). I trained the SVM classifier on theta ERSP patterns using a pair of odors (AP vs TP; HA vs TP; HA vs AP) (Figure 26A middle panel). Next, I tested the classifier on theta ERSPs for the test odor (Figure 26A right panel). Because this analysis is based on odor quality rather than odor valence or intensity (Figure 21), EEG signals may provide information for the quality of each specific odor. To verify if each odor is classified within the appropriate class, I also performed an odor similarity verification procedure (Figure 26B). When two test odor pairs are classified as the same test odor by the procedure outlined in Figure 26B, these two odors were considered similar. When the test odor does not classify both trained odor pairs, the test odor was defined as there was no simiarity between trained odors.

Before testing the classifier, I verified the accuracy (Table 7)and found that theta measurements are more than 97% accurate in total conditions based on verification results. Using these classifiers, I classified the test set to verify how they may separate. Across total participants, I found that AP and TP were classified as the same class in 50-100 ms, 150-200 ms, and 350-400 ms in theta (Figure 27A)and both 100-150 ms and 350-400 ms in gamma (Figure 27D). Specifically, in theta AP was classified as TP with 16.67% accuracy-by chance at 50-100 ms, and TP was classified as AP with the same level of accuracy. At 150-200 ms, AP was classified as TP with 29.17% accuracy and TP was classified as AP at 20.83% accuracy. HA was not classified as either AP or TP during these two time windows (accuracy-by chance=0%). At 350-400 ms, AP was classified as TP with 12.50% accuracy, and TP was classified as AP at 25.00% accuracy. However, HA was classified as TP with 8.33% accuracy. During other time windows, AP, TP, and HA and AP or HA and TP were not classified as the same. In gamma, AP was classified as TP with 16.67% accuracy-by chance at 100-150 ms and TP was classified as AP with 33.33% accuracy. In 350-400 ms, AP was classified as TP with 29.17% accuracy and TP was classified as AP at 25% accuracy. HA was not classified as either AP or TP during these two time windows (accuracy-by chance=4.17 and 0% each).

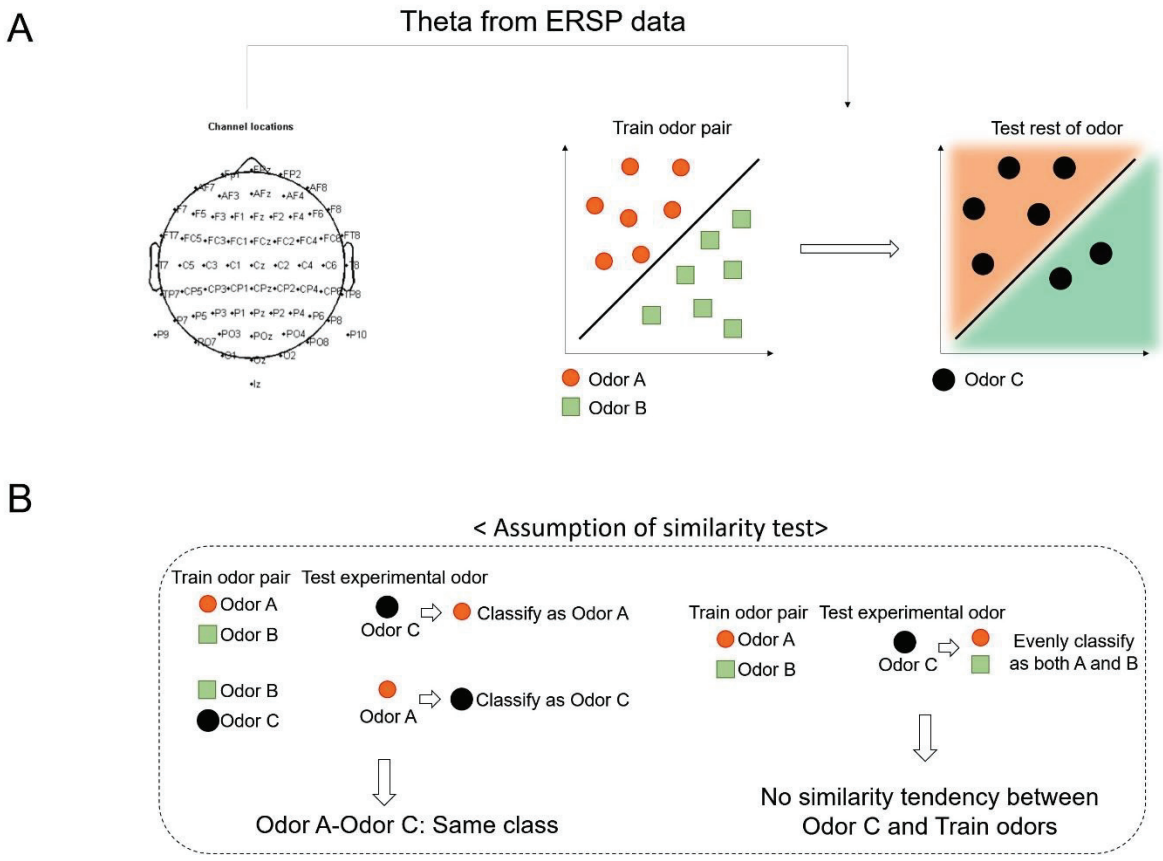



**Figure 26. Verifying pattern similarity by classification analysis.** A. Classification procedure designed to verify ensemble-pattern coding of odor category data. ERSP data from each odor were vetted during classification analysis. AP vs TP, HA vs AP, or HA vs TP were used for training data-set. HA, TP or AP were used for the test data-set. First, I trained a linear SVM using training data-sets to classify two odors (A, middle panel). Second, test data-sets were used to test how well the model can distinguish between two distinct odors that were classified as similar (A, right panel). B. Odor similarity verification procedure. When two test odors were classified as the same by the procedure outlined in A, these odors were paired in the same class. When test odors did not classify both trained odor pairs, the test odor was identified as there was no similarity between trained odors.

**Table 7. Classification model verification of theta.** Each number represents the accuracy of the classification model.

| Time (ms) | 0-50 | 50-100 | 100-150 | 150-200 | 200-250 | 250-300 | 300-350 | 350-400 |
|---|---|---|---|---|---|---|---|---|
| **AP vs TP** | 97.92 | 97.92 | 97.92 | 100 | 100 | 100 | 100 | 100 |
| **AP vs HA** | 97.92 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| **TP vs HA** | 100 | 97.92 | 100 | 100 | 100 | 100 | 100 | 100 |

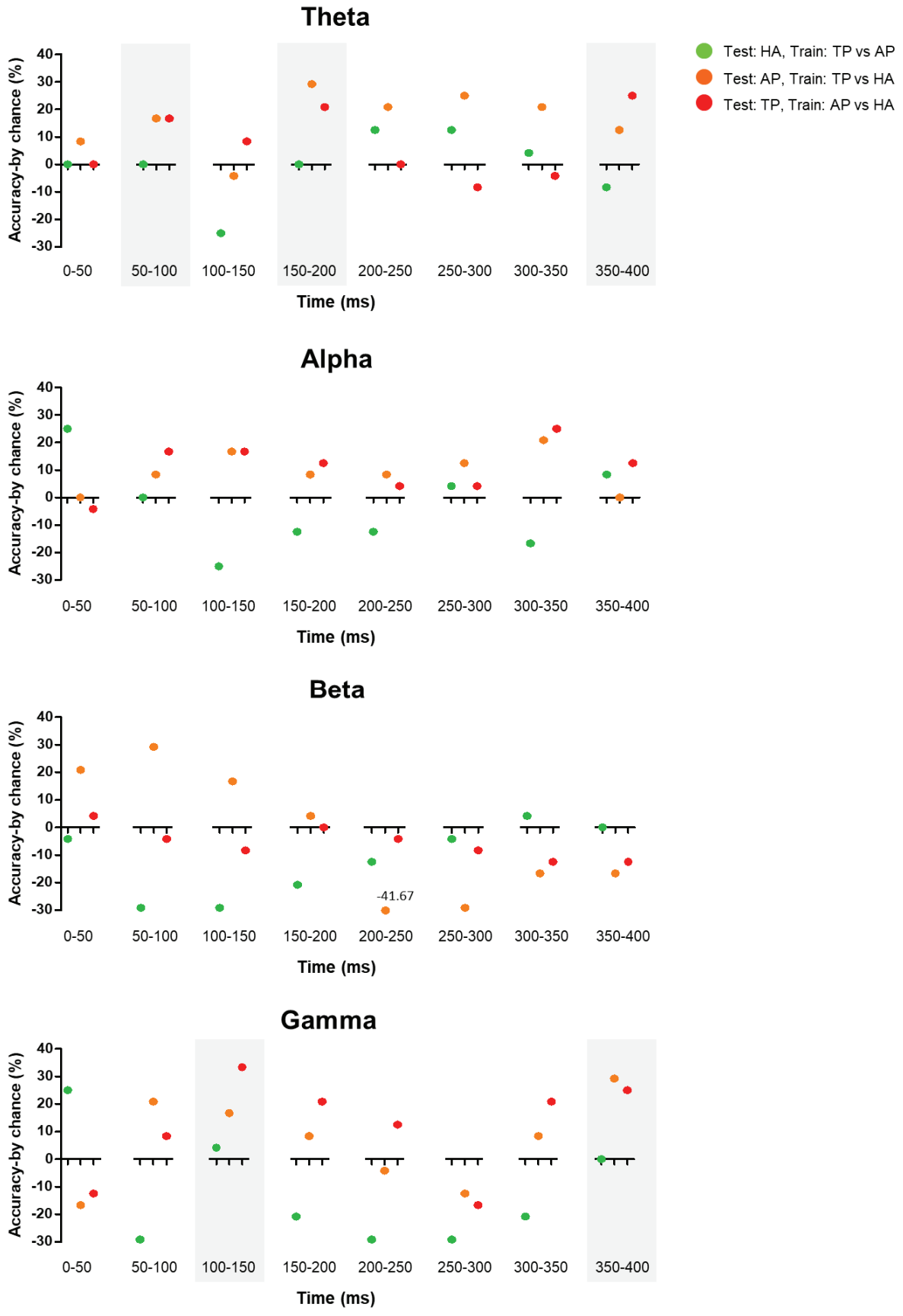



**Figure 27. Ensemble ERSP patterns between odors.** ERSP data were vetted using classification

analysis. The y-axis represents classification accuracy (accuracy-by chance) and the x-axis represents the time for each odor presentation. Green dots (HA test); orange dots (AP test); red dots (TP test). The red line represents accuracy-by chance=|10%| point. And the x-axis represents the time of each odor presentation. Green dots (HA test, positive direction indicated that HA classified as AP); orange dots (AP test, positive direction indicated that AP classified as TP); red dots (TP test, positive direction indicated that TP classified as AP. Gray box highlights results that varied within the same class across the experimental odors.

### 2.4 Verification of EEG source origination from olfactory-related brain areas

Within the 0-400 ms time period, overall odor stimulation conditions (HA, AP, and TP) originated from olfactory-related brain areas (Figure 28). Specifically, theta had the largest t-value in overall odor conditions. BA11 had the largest t-value and BA 27 also indicated a significant change during odor stimulations. In the case of BA25, AP and TP were not significantly different. In the case of beta and gamma, BA11 had the largest t-value. Although there was no significant difference between BA 25 and 27, BA11 was changed in overall odor conditions. I also found that time-varying theta activities occurred olfactory related area during odor stimulation (Figure 29). Specifically, superior temporal gyrus which located beside of piriform cortex activated at 64ms, and OFC activated at 152ms.

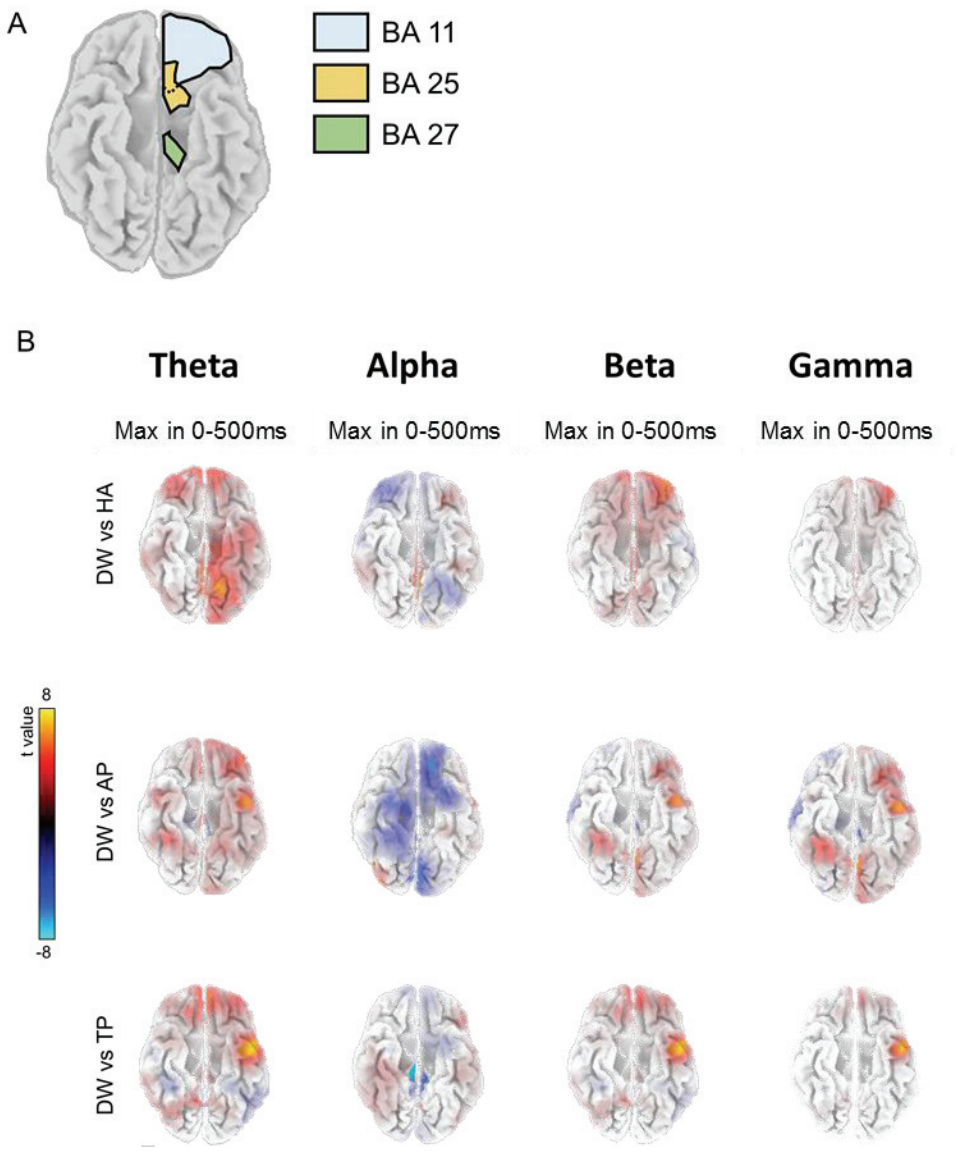
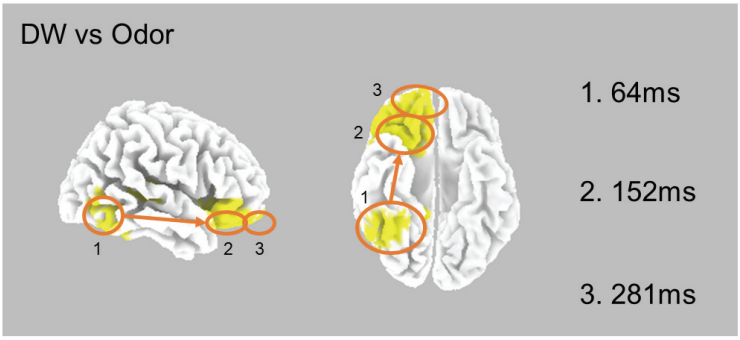



**Figure 28. ERSP source originated from olfactory-related brain areas.**

<Theta>




| | Brain regions | Brodmann area | Time (ms) |
|---|---|---|---|
| 1 | Fusiform gyrus | 37 | 64.45 |
| 2 | Superior temporal gyrus | 41 | 68.36 |
| 3 | Posterior cingulate | 29 | 138.67 |
| 4 | Orbital gyrus | 47 | 152.34 |
| 5 | Rectal gyrus | 11 | 281.25 |

**Figure 29. Time-varying theta activities during odor stimulation.** Theta activity locations estimated by sLORETA. Yellow colored areas represent significantly activated.

## 3 Discussion

The current results demonstrate that the brain may categorize odor information at specific periods during olfactory processing. I found that similar odors were able to induce similar theta activities at 100 ms. Specifically, brain activity following stimulation with two odors was perceived as similar (i.e., AP and TP). Odorants can be classified by ERSP and represent similar temporal and spatial patterns between similar odors, especially in theta (Figure 25 and Figure 27). Looking closer at the timeframe of these responses, spatial patterns of AP and TP clustered together with the same class at 100 ms and 150-200 ms (Figure 25). Moreover, multivariate patterns of theta and gamma also demonstrated that AP and TP induced similar ERSP patterns within 50—200 ms and 350-400 ms (Figure 27). These results suggest that odor information encoding may occur within 100 ms after odor stimulation and theta may be involved. Moreover, categorizing events can be represented by specific periods rather than overall periods.

My initial finding revealed that odor similarity information may be processed sufficiently within 100 ms (Figure 25 and Figure 27). According to a previous study, human subjects can discriminate odors within 420 ms [72] and behavioral evidence that respiration can be controlled in response to odors occurs within 160 ms [68]. Moreover, scalp EEG studies and amygdala iEEG studies report that olfactory-evoked potentials occur between 300–400 ms [81]. A study of early-stage odor processing using MEG reported that PC onset activity occurs around 80 ms [117]. Recently, evidence of olfactory-specific information being decoded as early as 110 ms following stimulation has been reported [56]. These studies suggest that odor categorical information may be processed within 500 ms and temporal odor processing may start between 80~300 ms. Although human studies do not provide direct neurological evidence of odor categorization within 500 ms, studies in animal models support the notion that odor information can be processed within 200 ms [1,28,123,127]. In previous ERP studies, N1 was suggested at 200–700 ms [27,40] and recent EEG studies also report that OB activation was observed at 100-200 ms [54]. However, odor categorization can be occurred before it is observed in N1, and the OB can be modulated from the top down. Based on these studies, the current data provides evidence that odor information can be resolved within 100 ms.

Increasing categorization accuracy between AP and TP at 150-200 ms and 350-400 ms may suggest that a further similarity categorizing step exists, and possibly even two more steps. I can assume that this step may depend on activity in a specific brain region as I found a similar spatial pattern during the 150-200 ms time window (Figure 25). These results are also in line with previous human studies. A study using MEG reported that the primary olfactory cortex (PC, amygdala, and entorhinal cortex), parahippocampal gyrus, and OFC show increasing activity between 150-200 ms [117]. Moreover, intracortical EEG findings suggest increasing PC activities were also observed from 110 ms and were constantly increasing near 500wms [56]. These previous studies suggest that the activities of primary and secondary olfactory cortexes increase at 150-200 ms. Interestingly, I also found similar AP and TP activities at 350-400 ms, though there were no spatial pattern similarities between AP and TP. I cannot conclude that there is no categorizing event through spatial patterns due to the low spatial resolution of EEG. However, these findings I suggest that odor similarity categorization events may occur at 350-400 ms with more systemically coordination of brain areas than the early response period (50-200 ms).

Notably, theta waves were the most representative frequency band in the current study. Although there is comparatively less evidence of theta activity in olfactory processing, theta waves showed the highest reliability and most precise performance supporting my hypothesis, which suggests a relativeness of odor categorization (Figure 23). Growing evidence supports the idea that theta waves are related to olfaction. Theta waves are altered when stimulation is conducted with distinct odors [70,82] and are associated with hippocampus activities [37,90]. Using intracortical EEG, theta waves were induced by odor stimulation in the PC, suggesting that theta waves may originate from odor processing [56]. Although the precise role of theta waves in olfaction remains unclear, recent studies and findings in the current study suggest that theta waves may be related to odor processing.

Other frequency bands are also noteworthy as beta and gamma were known to have an essential role in olfactory processing. Although they may represent the classification of each experimented odor (Figure 27), which is less supportive of my hypothesis. For these results, I concentrated on two possible factors. First, I performed my experiments with humans. The relationship between these frequencies and odor processing is well documented in animal studies. Gamma waves have been noted in field potential recordings from the OB, PC, and OFC [2,11,64,91], and beta oscillation has been described in

olfactory-related brain areas (i.e., OB, PC, entorhinal cortex) after stimulation [22,124,140]. However, these observations have not been clearly characterized in humans. No significant enhancement of gamma waves in the PC has been reported [56] and though gamma can be one indicator of odor recognition, evidence of a role involving odor similarity categorization is still lacking [6]. Recent studies have suggested that gamma may represent olfactory processing specifically in the OB, and that gamma does not represent odor identity under experiment conditions [54]. Few studies have reported human intracranial recording studies of the amygdala—an area that is known to play a less significant role in odor categorization [81]. Thus, beta and gamma waves may be less related to odor similarity categorization in humans compared to animal studies. Another possible explanation relates to my experimental design. According to previous studies, beta and gamma waves were revealed to play a role in odor discrimination in olfactory memory tasks [7,66,86] and most studies have been conducted using intracranial recordings. In contrast, I conducted natural respiration studies and stimulated responses to odor without any additional tasks (Figure 20). I also performed experiments with a scalp EEG device which may result in differences in intracranial recordings, though both have related signal sources and activities [15]. The high frequency of EEG recordings has a weaker ability to detect signals by scalp EEG when compared to lower frequency signals [26]. This different approach to analysis may attenuate the weight of beta and gamma.

Here, I provide essential evidence for the temporal resolution of odor information. Although previous studies have similarly reported information related to the temporal activities of the brain during odor stimulation, prior studies have not clearly verified specifically when odor information is processed. In the current study, I verified when and how odor information can be categorized during processing. Odors that are perceived as similar induced systemically similar brain activity for both spatial and temporal theta patterns and these patterns occurred during specific periods including the 100 ms time period. These findings suggest that theta waves are correlated with odor information processing in humans and categorizing odor information may occur during a specific time rather than during an overall period. Because of the limited number of odors examined in this study, I cannot broadly conclude whether these brain signals play a role in differentiating between odor similarities. However, my study provides essential evidence of how the brain differentiates among odors during varying time points, specifically during early olfactory processing.

# VII. Characterization of odor quality perception using odor profiling

## 1 Background

In the current study, I established a characterizing method to measure odor quality. Although I verified that olfactory processing signals can be measured by EEG, the object of my thesis is to add a greater understanding of odor information processing. In addition to screening odor categorization features from EEG, it is necessary to characterize odor similarity at the behavioral level as well. From a physiological view, perceiving odor begins with activating a specific odorant receptor (OR) repertoire set in response to volatile chemicals[35,62,83]. This odor information processes and categorizes each specific odor quality in the brain [44,99,120,141]. Previous studies suggest that detected odors are processed using specific information that may relate to the quality of odor perception. Moreover, coding to categorize an odor and to identify an odor is located in different brain regions [48,49], a finding supported by findings in the current thesis (studies 1 and 2). This physiological evidence also suggests that odor quality can be influenced by behavioral output, and findings from behavioral and survey studies support the possibility that odor quality can be quantified. Increasing evidence of high-performance odor discrimination ability suggests that humans exhibit high performance in categorizing odors, despite possessing low performance in identifying and naming those odors [16,73,77,133].

Despite this evidence, quantifying odor quality is known to be a challenging area of study. One of the reasons is that odor perception has a multidimensional axis with less evidence for perceptual space [20,41]. Although physiological evidence suggests precise abilities for odor quantification, more evidence is needed to predict the multidimensional axis of odor perception. Measuring odor discrimination is also wrought with challenges for quantifying odor. Because the discriminating odor task does not represent the multidimensional axis of odor, there are no a priori limits on describing odor differences. Based on these observations, odor profiling is the proper quantification method as it measures the multidimensional axis of odor by rating various descriptors [33,131].

Profiling odors is also challenging as profiling happens using a subjective rating of odor quality descriptors. Odor responses can be different based on individual experience, so odor profiles can vary by condition, despite stimulating with the same odor [5,47,51,80], however, both training and experience increase discrimination accuracy of the components in odor mixtures[80]. Additionally, cultural variations cause different responses to odor categorization[5,24,132]. Moreover, presenting verbal cues with odor stimulation alters odor responses, suggesting that odor perception is significantly influenced by verbal labeling [47]. These studies suggest that subjective rating can be influenced by individual experience and that measuring odor profiles is less accurate than other methods such as behavioral or neurological approaches [16,44].

Based on previous studies, it is necessary to characterize alterations in odor profiles for stimulating odors by outlining subjective rating conditions. I hypothesize that odor may have a specific primary odor quality which is minimally altered by subjective rating conditions. Although odor responses can be altered in some way with respect to odor quality factors under verbal cue conditions, invariant or less variant parts of odor profiling may exist as implied by previous studies [48]. To address this hypothesis, I characterized altering odor quality patterns in response to the same odor under verbal cues based on previous research which suggests that other sensory cues can influence odor quality perception [32,47,84,93,137]. For example, the perceived intensity of food odors was increased when presented in colored liquid [137], and fragrance pleasantness and sweetness perception were altered when supplied with or without brand labels [93]. Furthermore, odor responses were significantly influenced by different verbal cues which were both positive and negative [47,84]. Thus, I measured odor quality using profiling methods while presenting different verbal cues to observe altering patterns of odor quality. I used isovaleric acid (IVA) and heptanol (Hep) for this experiment. I assessed odor responses to IVA with and without verbal cues, in addition to Hep. Because IVA is known as an ambiguous odor, inducing significantly different responses even in the brain [29], it can be used as a model for characterizing alterations of odor profiles. Hep is used for a negative control due to its drastically different odor quality compared to IVA. Because measurement of alterations of IVA odor profiles was performed based on verbal cues, Hep was used as the standard for measuring the range of alteration. Although it is hard to understand the extent to which odor profiles are altered by conditions, predictions regarding the degree of alteration can be carried out using a negative control such as Hep.

## 2 Results

### 2.1 Experimental design for sorting objective-rated odor descriptors.

The experiment began with the odor quality-rating test of a verbal cue-odor stimulation (Figure 30). Odors were presented while verbal cues were displayed on the screen. For group 1, IVA and Hep were presented with a blank screen (B-IVA, B-Hep) at different times. For group 2, IVA was presented with the “Cheese” verbal cue (C-IVA). For group 3, IVA was presented with the “Vomit” verbal cue (V-IVA). A total of 146 descriptors on odor quality were given to evaluate odor qualities (Table 1), and six questionnaires were provided to evaluate additional odor responses, including identifying the odor, pleasantness, intensity, familiarity, edibility, and relaxing effect (Table 2). Discussion among the participants was not allowed until the end of the experiment.

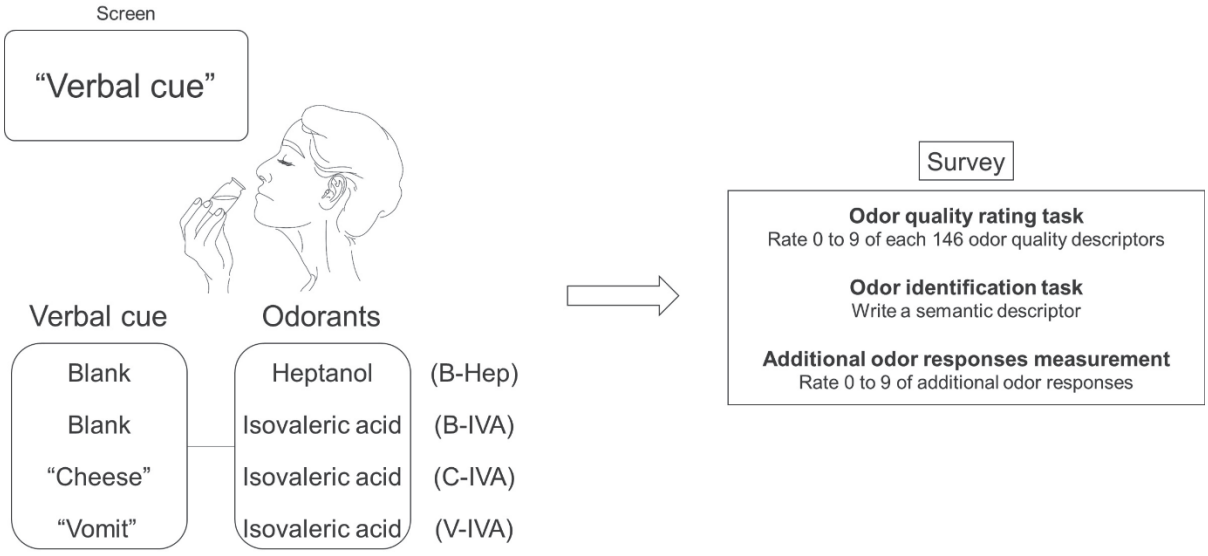



**Figure 30. Experimental design.** Odor stimulations were given while presenting verbal cues on the screen. Blank-Heptanol (B-Hep), blank-isovaleric acid (B-IVA), cheese-isovaleric acid (C-IVA) and vomit-isovaleric acid (V-IVA) were the stimulation conditions. The survey was performed while presenting stimulations.

Participants were randomly divided into three groups (Table 8). Group 1 (n=32, 14 females, 18 males) was subjected to stimulation by IVA and Hep with a blank screen. Group 2 (n=32, 10 females, 22 males) was subjected to stimulation by IVA with the “cheese” verbal cue on the screen. Group 3 (n=32, 8 females, 24 males) was subjected to stimulation by IVA with the “vomit” verbal cue on the screen. No significant differences were found between groups with respect to age, odor threshold, or odor discrimination based on the results of a one-way ANOVA.

**Table 8. Information on odor ability in each participants group (mean ± SD)**

| | Age | Threshold | Discrimination |
|---|---|---|---|
| **Group 1 (B-IVA, B-Hep)** | 21.00±2.83 | 6.47±2.02 | 10.28±1.05 |
| **Group 2 (C-IVA)** | 21.31±3.00 | 5.97±1.99 | 10.16±1.14 |
| **Group 3 (V-IVA)** | 20.75±1.55 | 5.94±2.00 | 10.25±1.16 |
| **Statistics (p value)** | 0.68 (ns) | 0.49 (ns) | 0.90 (ns) |

Participants' information regarding age, odor threshold, and odor discrimination for each group. A one-way ANOVA was performed for statistical comparison.

2.2 Verbal cues altered odor quality pattern.

First, each stimulation condition exhibited different odor quality patterns based on odor profiling as outlined in previous studies [32,47,84,93,137]. I performed odor descriptor survey tasks of B-IVA (verbal cue: blank, odor: IVA), C-IVA (verbal cue: “cheese”, odor: IVA), and V-IVA (verbal cue: “vomit”, odor: IVA) conditions to examine the effect of verbal cues. The B-Hep (verbal cue: blank, odor Hep) condition was used to define the standard for alterations of odor quality patterns as Hep differs distinctly from IVA based on smell (Figure 31 A). To verify the overall influence of each condition, I compared IVA conditions to the B-Hep condition by two-way ANOVA. Each rating value for descriptors was used as a row factor and each condition was used as a column factor. I found significant differences between B-IVA, V-IVA, C-IVA, and B-Hep ($F[3,18104]=67.84$, $p<0.0001$, $p\eta2=0.011$, Two-way ANOVA) (Figure 31B). C-IVA and V-IVA had significantly higher survey rating values than B-IVA, although these conditions used the same odor stimulation ($p<0.0001$, Bonferroni post hoc test each). C-IVA was also significantly different from V-IVA ($p<0.0001$, Bonferroni post hoc test). B-Hep was the second condition that differed from B-IVA. To verify the distance between conditions in odor quality space, a multivariate analysis (PCA), was performed. Results of the multivariate analysis suggest that the odor quality pattern indicated C-IVA had similar distances as B-Hep from B-IVA. Specifically, the spread patterns in odor quality space showed that components of C-IVA were located away from the center of B-IVA (Figure 31C). Although the density of elements was lower than B-Hep, the centroid of C-IVA was spread in a similar distance pattern to B-Hep. In contrast, the spread pattern of V-IVA was similar to B-IVA. Finally, I performed a cluster analysis between conditions (Figure 31D) to evaluate the distance. B-Hep was rated to be the most different class compared to B-IVA (186.67 distance), and C-IVA was rated to be a similar distance from B-IVA (176.59 distance). V-IVA was rated in the same class as B-IVA (161.50 distance).

To verify alterations in odor identification and pleasantness, additional survey tasks were performed. I found that C-IVA had significantly different identification and pleasantness patterns from B-IVA, although V-IVA was similar to B-IVA (Figure 32 and Table 9). Pleasantness was also shown to be significantly different between C-IVA and V-IVA ($T[62]=2.82$ $p=0.0065$). These data suggest that verbal cues induce alterations of odor quality patterns in IVA.

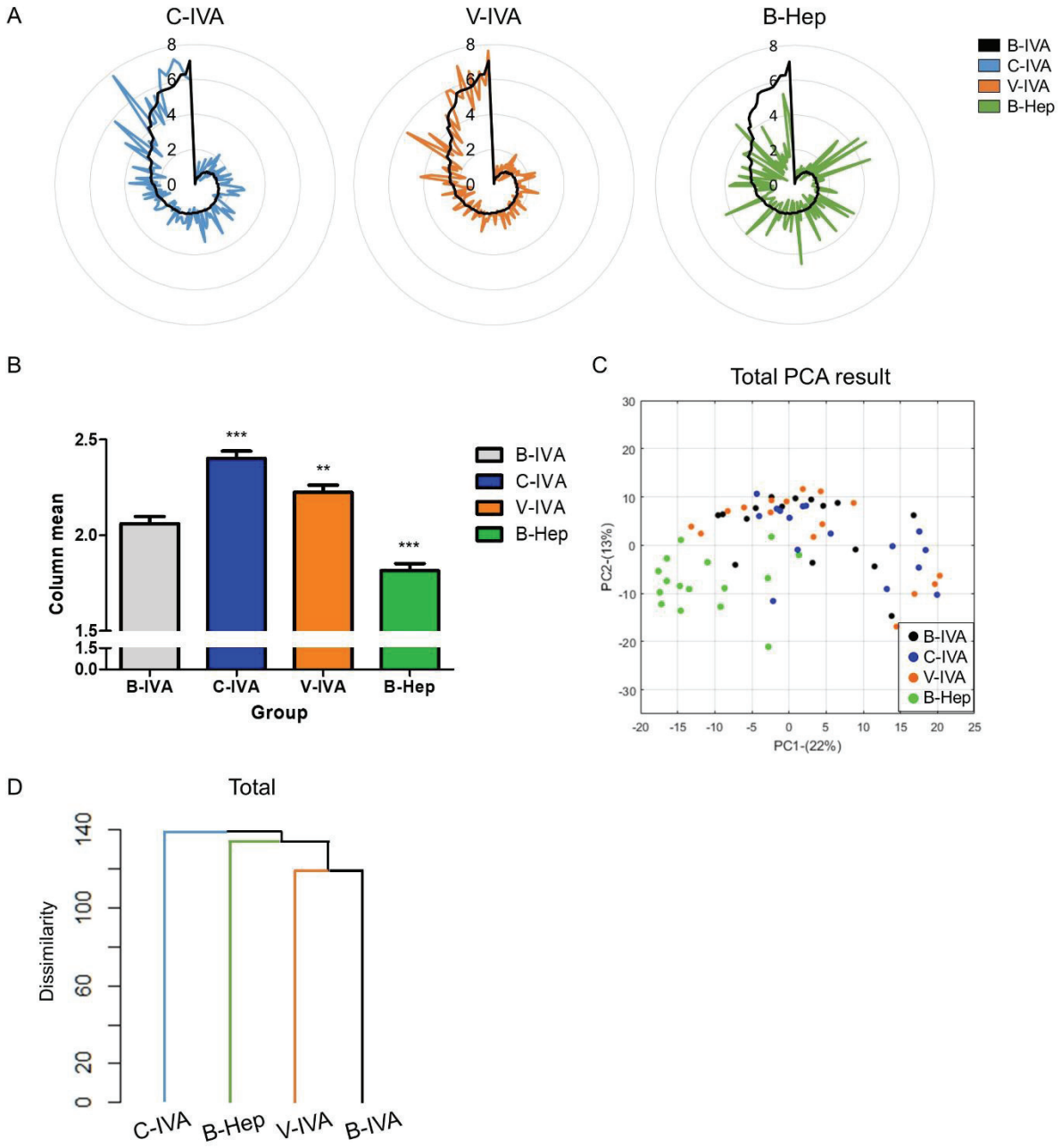



**Figure 31. Patterns of odor descriptors among the different stimulation conditions.** A. Descriptor survey rates of each stimulation condition. 146 values are presented as a radar chart in counter-clockwise descending order by B-IVA. The black line is B-IVA, blue is C-IVA, orange is V-IVA, and green is B-Hep. B. Verification of differences by stimulation conditions. Based on results of a two-way ANOVA, C-IVA, V-IVA, and B-Hep were significantly different from B-IVA. Two cued IVA conditions (C-IVA, V-IVA) were also significantly different from each other. C. Odor quality space comprised principal component 1 (PC1: 22%) and principal component 2 (PC2: 14%). Each dot was projected from each participant's 146 descriptor values. D. Verification of similarity between stimulation conditions. Compared with B-IVA, V-IVA was next to B-IVA (161.50 distance), but C-IVA was rated as more dissimilar (176.59 distance). B-hep was rated as the most dissimilar condition compared to B-IVA (186.67 distance).

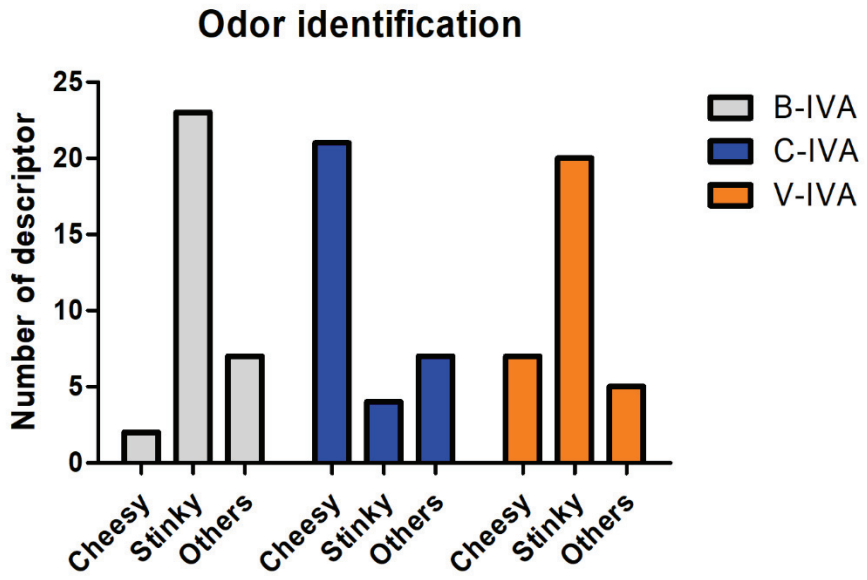



**Figure 32. Odor identification.** Odor identification of total participants in each group. Y-axis represents a summation of participants' number which is obtained from the odor identification task (S2 Table). The x-axis represents semantic descriptors that participants used. 'Parmesan cheese', 'cheddar cheese', 'blue cheese' regarded as 'cheesy'. 'Vomit', 'stinky foot', 'sweat' regarded as 'stinky'. B-IVA had a higher value on 'stinky' compared to 'cheesy', C-IVA had the highest value for 'cheesy' and V-IVA had the highest value for 'stinky'. The most frequent semantic descriptor that was provided by participants was 'vomit' in B-IVA (vomit: 10, stinky foot: 5, sweat: 8), 'cheese' in C-IVA (cheese: 15, parmesan cheese: 3, cheddar cheese: 3), and 'vomit' in V-IVA (vomit: 13, stinky foot: 4, sweat: 3).

**Table 9. Comparison of odor responses between B-IVA and other stimulation conditions.** Each stimulation condition was compared with B-IVA odor response values.

| | B-IVA vs C-IVA | | B-IVA vs V-IVA | | B-IVA vs B-Hep | |
|---|---|---|---|---|---|---|
| | ***p-value*** | ***t-value*** | ***p-value*** | ***t-value*** | ***p-value*** | ***t-value*** |
| **Pleasantness** | <0.001 (***) | 3.54 | 0.72 | 0.35 | <0.001 (***) | 9.83 |
| **Intensity** | 0.94 | 0.078 | 0.25 | 1.17 | 0.067 | -1.86 |
| **Familiarity** | 0.26 | 1.14 | 0.55 | 0.60 | 0.22 | 1.23 |
| **Edibility** | <0.001 (***) | 5.081 | 0.23 | 1.21 | <0.001 (***) | 4.54 |
| **Relaxing effect** | 0.38 | 0.88 | 0.12 | -1.57 | <0.001 (***) | 6.43 |

***two tail t-test, DF=62*** **** (p<0.05), ** (p<0.01), *** (p<0.001)***

2.3 Odor quality patterns are altered depending on rating score of descriptors by conditions.

To examine alterations specifically in response to verbal cues and odors, I compared total descriptor ratings from stimulation conditions to B-IVA. I arranged descriptors in descending order with B-IVA rating values (Table 10). Descriptors of other stimulation conditions were arranged in the same manner as for B-IVA arrangement. Figure 33 shows deviation between each stimulation condition and B-IVA. When rating score patterns of C-IVA, V-IVA, and B-Hep to B-IVA were compared, I found that both C-IVA and V-IVA have similar patterns as B-IVA on total descriptors. However, the B-Hep condition showed different patterns, especially in the upper 25% of data values. In contrast, the lower 75% of data values exhibited less difference among conditions. These results suggest that the upper 25% of data values represent odor quality descriptors more specific to IVA rather than Hep. Based on these results, I defined 'UD (upper 25% odor quality descriptors)' as the upper 25% of B-IVA odor quality descriptors and 'LD (lower 75% odor quality descriptors)' as the lower 75% of B-IVA odor quality descriptors. Table 11 and Table 12 shows the upper 25% odor quality descriptors of B-IVA and the upper 25% odor quality descriptors of C-IVA, V-IVA, and B-Hep, respectively.

**Table 10. Descending order of B-IVA odor descriptors**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | sickening | 31 | medicinal | 61 | cooked vegetables | 91 | strawberry-like | 121 | cedarwood-like |
| 2 | aromatic | 32 | wet wool, wet dog | 62 | chalky | 92 | kerosene | 122 | lavender |
| 3 | stale | 33 | oily, fatty | 63 | honey-like | 93 | spicy | 123 | varnish |
| 4 | dirty linen-like | 34 | rubbery (new rubber) | 64 | burnt rubber-like | 94 | peach (fruit) | 124 | green pepper |
| 5 | rancid | 35 | sulphidic | 65 | sauerkraut-like | 95 | crushed-weeds | 125 | cherry (berry) |
| 6 | Sweaty | 36 | fishy | 66 | tar-like | 96 | grapefruit | 126 | fried chicken |
| 7 | putrid, foul, decayed | 37 | cork-like | 67 | almond-like | 97 | disinfectant, carbolic | 127 | burnt milk |
| 8 | musty, earthy, moldy | 38 | banana-like | 68 | beery (beer-like) | 98 | bean-like | 128 | oak wood, cognac-like |
| 9 | like ammonia | 39 | floral | 69 | sooty | 99 | burnt candle | 129 | raw cucumber-like |
| 10 | fecal (like manure) | 40 | raisins | 70 | paint-like | 100 | soupy | 130 | minty, peppermint |
| 11 | heavy | 41 | fruity (other) | 71 | rose-like | 101 | black pepper-like | 131 | fresh green vegetables |
| 12 | animal | 42 | turpentine (pine oil) | 72 | crushed-grass | 102 | meaty (cooked, good) | 132 | celery |
| 13 | sewer odor | 43 | metallic | 73 | bakery (fresh bread) | 103 | alcohol-like | 133 | malty |
| 14 | urine-like | 44 | woody, resinous | 74 | soapy | 104 | like gasoline, solvent | 134 | dry, powdery |
| 15 | fermented (rotten) fruit | 45 | sweet | 75 | lemon (fruit) | 105 | apple (fruit) | 135 | violets |
| 16 | sour | 46 | garlic, onion | 76 | household gas | 106 | cologne | 136 | molasses |
| 17 | cheesy | 47 | hay | 77 | cool, cooling | 107 | caramel | 137 | eucalyptus |
| 18 | light | 48 | raw potato-like | 78 | herbal, green, cut grass | 108 | perfumery | 138 | musk-like |
| 19 | sour milk | 49 | coconut-like | 79 | grape-juice-like | 109 | coffee-like | 139 | etherish, anaesthetic |
| 20 | cadaverous, like dead animal | 50 | fruity (citrus) | 80 | tea-leaves-like | 110 | pear (fruit) | 140 | creosote |
| 21 | cat-urine-like | 51 | like mothballs | 81 | - 94 -ipper (smoked fish) | 111 | cantaloupe, honey dew melon | 141 | laurel leaves |
| 22 | leather-like | 52 | seasoning (for meat) | 82 | orange (fruit) | 112 | cinnamon | 142 | dill-like |
| 23 | warm | 53 | wet paper-like | 83 | eggy (fresh eggs) | 113 | popcorn | 143 | caraway |
| 24 | chemical | 54 | bark-like, birch bark | 84 | buttery (fresh) | 114 | seminal, sperm-like | 144 | camphor-like |
| 25 | mouse-like | 55 | grainy (as in grain) | 85 | vanilla-like | 115 | nail polish remover | 145 | geranium leaves |
| 26 | sharp, pungent, acid | 56 | nutty (walnut, etc.) | 86 | peanut butter | 116 | fragrant | 146 | incense |
| 27 | mushroom-like | 57 | stale tobacco smoke | 87 | cardboard-like | 117 | like burnt paper | - | - |
| 28 | bitter | 58 | pineapple (fruit) | 88 | fresh tobacco smoke | 118 | clove-like | - | - |
| 29 | yeasty | 59 | like cleaning fluid (carbona) | 89 | anise (licorice) | 119 | maple (as in syrup) | - | - |
| 30 | rope-like | 60 | like blood, raw meat | 90 | burnt, smoky | 120 | chocolate | - | - |

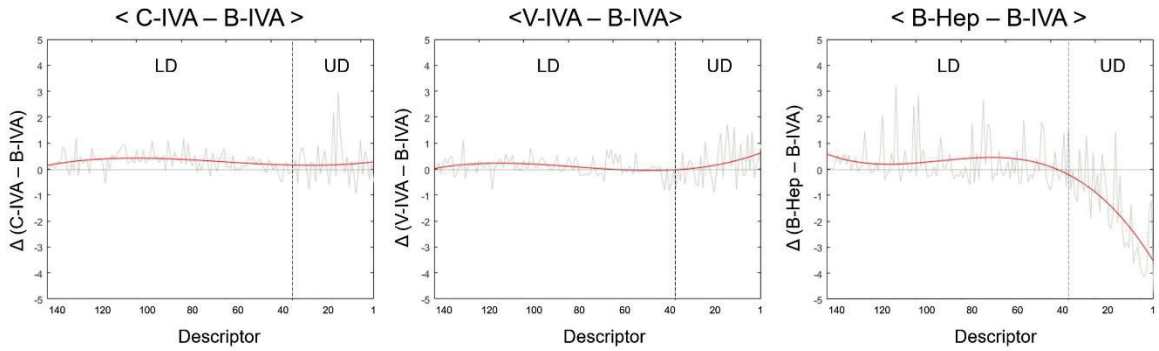



**Figure 33. Different alteration patterns of odor descriptors depending on the descriptor rating score.** Change of survey rate between B-IVA and other conditions. X-axis represents descending order of descriptors from B-IVA odor quality rating (S4 Table). Y-axis represents deviation of odor quality rating for each stimulation condition (C-IVA, V-IVA, B-IVA) and B-IVA condition. Grey line indicates changing values. Orange line indicates curve fitting of the grey line. Dotted line indicates boundary between upper 25% of data (UD) and the rest of the data (LD: lower 75% of data).

**Table 11. List of upper 25% odor quality descriptors in B-IVA**

| | | | | | |
|---|---|---|---|---|---|
| 1 | sickening | 16 | sour | 31 | medicinal |
| 2 | aromatic | 17 | cheesy | 32 | wet wool, wet dog |
| 3 | stale | 18 | light | 33 | oily, fatty |
| 4 | dirty linen-like | 19 | sour milk | 34 | rubbery |
| 5 | rancid | 20 | cadaverous, like dead animal | 35 | sulphidic |
| 6 | sweaty | 21 | cat-urine-like | 36 | fishy |
| 7 | putrid, foul, decayed | 22 | leather-like | 37 | cork-like |
| 8 | musty, earthy, moldy | 23 | warm | 38 | banana-like |
| 9 | like ammonia | 24 | chemical | | |
| 10 | fecal (like manure) | 25 | mouse-like | | |
| 11 | heavy | 26 | sharp, pungent, acid | | |
| 12 | animal | 27 | mushroom-like | | |
| 13 | sewer odor | 28 | bitter | | |
| 14 | urine-like | 29 | yeasty | | |
| 15 | fermented (rotten) fruit | 30 | rope-like | | |

List of upper 25% odor descriptors of B-IVA. This list is defined as ‘UD’.

**Table 12. List of upper 25% odor quality descriptors in C-IVA, V-IVA and B-Hep.**

<C-IVA>

| | | | | | |
|---|---|---|---|---|---|
| 1 | aromatic | 16 | urine-like | 31 | fishy |
| 2 | putrid, foul, decayed | 17 | like ammonia | 32 | spicy |
| 3 | cheesy | 18 | animal | 33 | mouse-like |
| 4 | sickening | 19 | warm | 34 | buttery (fresh) |
| 5 | rancid | 20 | sharp, pungent, acid | 35 | cat-urine-like |
| 6 | dirty linen-like | 21 | cadaverous, like dead animal | 36 | medicinal |
| 7 | sour milk | 22 | oily, fatty | 37 | soupy |
| 8 | stale | 23 | leather-like | 38 | beery (beer-like) |
| 9 | sweaty | 24 | light | | |
| 10 | musty, earthy, moldy | 25 | rubbery | | |
| 11 | sour | 26 | chemical | | |
| 12 | sewer odor | 27 | yeasty | | |
| 13 | heavy | 28 | bitter | | |
| 14 | fecal (like manure) | 29 | wet wool, wet dog | | |
| 15 | fermented (rotten) fruit | 30 | peanut butter | | |

<V-IVA>

| | | | | | |
|---|---|---|---|---|---|
| 1 | sickening | 16 | urine-like | 31 | beery (beer-like) |
| 2 | rancid | 17 | cheesy | 32 | paint-like |
| 3 | aromatic | 18 | cadaverous, like dead animal | 33 | stale tobacco smoke |
| 4 | putrid, foul, decayed | 19 | sharp, pungent, acid | 34 | rubbery |
| 5 | stale | 20 | animal | 35 | tar-like |
| 6 | sewer odor | 21 | bitter | 36 | burnt rubber-like |
| 7 | fecal (like manure) | 22 | light | 37 | like gasoline, solvent |
| 8 | Sweaty | 23 | mouse-like | 38 | leather-like |
| 9 | sour | 24 | cat-urine-like | | |
| 10 | dirty linen-like | 25 | fishy | | |
| 11 | musty, earthy, moldy | 26 | chemical | | |
| 12 | heavy | 27 | oily, fatty | | |
| 13 | sour milk | 28 | wet wool, wet dog | | |
| 14 | like ammonia | 29 | warm | | |
| 15 | fermented (rotten) fruit | 30 | medicinal | | |

<B-Hep>

| | | | | | |
|---|---|---|---|---|---|
| 1 | aromatic | 16 | orange (fruit) | 31 | herbal, green, cut grass |
| 2 | light | 17 | lavender | 32 | musty, earthy, moldy |
| 3 | fragrant | 18 | sour | 33 | tea-leaves-like |
| 4 | cool, cooling | 19 | bitter | 34 | wet paper-like |
| 5 | cologne | 20 | soapy | 35 | like gasoline, solvent |
| 6 | stale | 21 | rubbery | 36 | cork-like |
| 7 | medicinal | 22 | heavy | 37 | kerosene |
| 8 | perfumery | 23 | warm | 38 | burnt rubber-like |
| 9 | like mothballs | 24 | sweet | | |
| 10 | floral | 25 | grapefruit | | |
| 11 | like cleaning fluid (carbona) | 26 | minty, peppermint | | |

| | | | | | |
|---|---|---|---|---|---|
| 12 | fruity (other) | 27 | sharp, pungent, acid | | |
| 13 | fruity (citrus) | 28 | chalky | | |
| 14 | lemon (fruit) | 29 | paint-like | | |
| 15 | chemical | 30 | leather-like | | |

List of upper 25% odor descriptors of C-IVA, V-IVA, and B-Hep. Grey colored cells represent descriptors on the list which did not appear on the list of UD (Table 11: the upper 25% of B-IVA odor quality descriptors).

### 2.4 UD descriptors are less altered compared to LD descriptors in IVA

To verify differences between experimental conditions based on the categories of UD and LD, I compared differences of odor profiles between B-IVA, C-IVA, V-IVA, and B-Hep by separating UD and LD (Figure 34A, B, and Table 13). In UD, I found no significant differences among IVA conditions but observed significant differences compared to B-Hep. Differences among the conditions were verified ($F[3,4712]=181.91$, $p<0.0001$, ${}_p\eta^2=0.10$, Two-way ANOVA), but results of post-hoc testing suggested that only B-Hep was rated significantly lower compared to each IVA condition ($p<0.0001$, Bonferroni post hoc test each). In LD, I found significant differences between IVA conditions and the B-Hep condition. Differences among conditions were verified ($F[3,13392]=35.60$, $p<0.0001$, ${}_p\eta^2=0.0079$, Two-way ANOVA), and results from post-hoc tests suggested that all conditions were significantly different from B-IVA, except the V-IVA condition (B-IVA vs C-IVA, $p<0.0001$ | B-IVA vs V-IVA, $p=0.061$ | B-IVA vs B-Hep, $p<0.0001$ | C-IVA vs V-IVA, $p<0.0001$, Bonferroni post hoc test each). C-IVA and B-Hep rated significantly higher than B-IVA. These results suggest no significant differences between IVA conditions in UD, but significantly reversed patterns were observed in LD. Moreover, B-Hep was significantly different from total IVA conditions in UD but differences were reduced in LD.

To verify alterations in the distance between stimulation conditions in the odor quality space, I performed a multivariate analysis to observe spread patterns. I found that dots of IVA conditions in UD were uniformly distributed, whereas B-Hep condition dots were scattered to the left side of IVA conditions dots (Figure 34 C). In LD, C-IVA dots were scattered to the right side of B-IVA and V-IVA (Figure 34D). Cluster analysis showed a more precise alteration distance between conditions. I found that UD represented more similar IVA odor qualities compared to LD (Figure 34E and F). Specifically, IVA conditions were clustered in a similar class in UD (Figure 34E). B-IVA and V-IVA were clustered in the same class, and C-IVA was clustered in the next closest class. B-Hep was clustered in the most distant class in UD. In contrast, IVA clusters were dissembled in LD (Figure 34F). C-IVA became further from V-IVA and B-IVA, at an even greater distance than B-Hep which showed different odor quality profiles. These results suggest that UD represents more consensus IVA odor quality than LD.

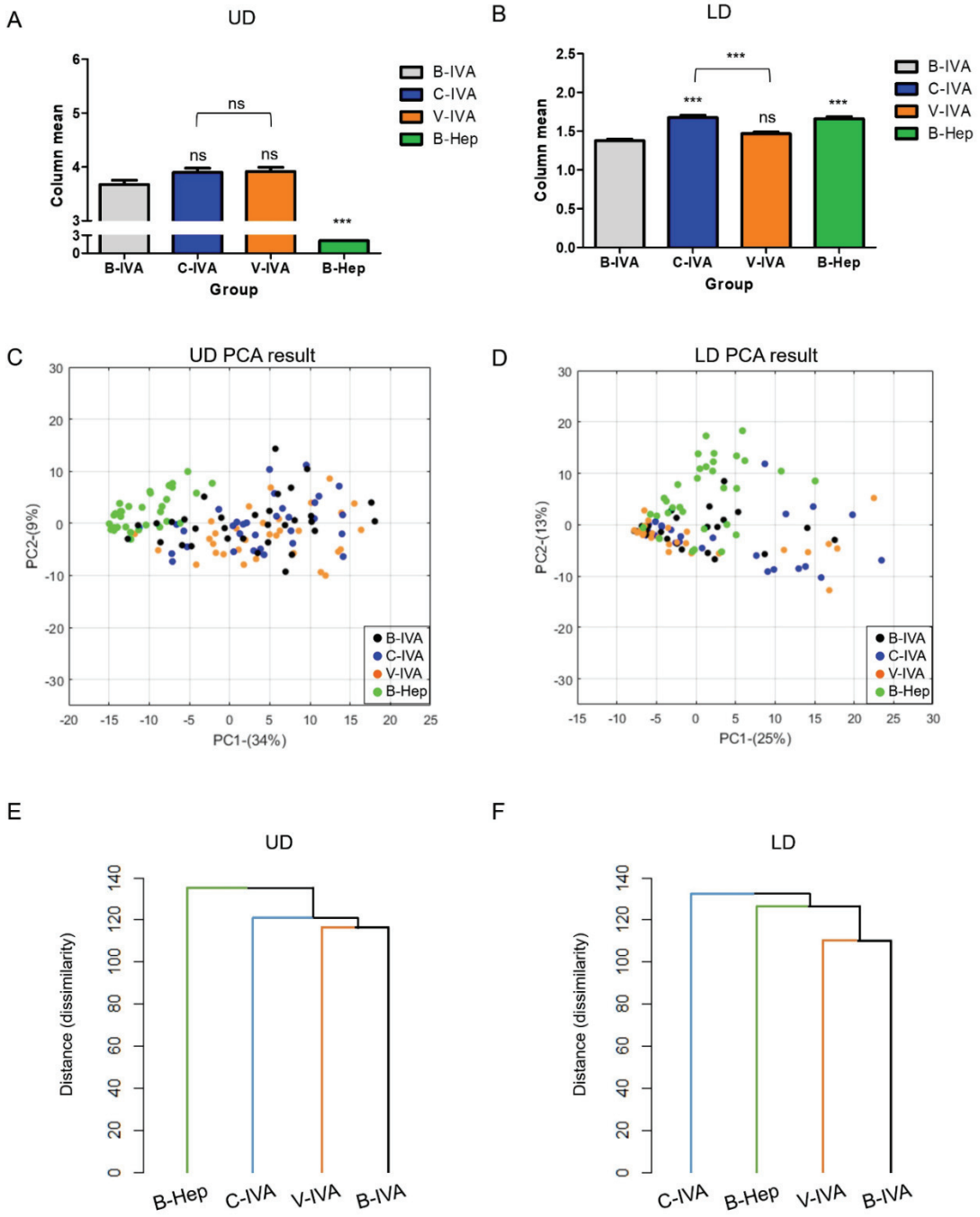



**Figure 34. Different patterns of odor descriptors between two datasets separated by first quartile points.** (A-B) Verification of differences in stimulation conditions. A. Based on a two-way ANOVA, B-Hep was significantly different from B-IVA but C-IVA and V-IVA had no differences. B. Based on a two-way ANOVA, C-IVA, V-IVA, and B-Hep were significantly different from B-IVA. C-D. Odor quality space comprised PC1 (C: 34%, D: 25%) and PC2 (C: 9%, D: 13%). Each dot was projected from each participant's 37 descriptor values in C. and 109 descriptor values in D. E-F. Verification of similarity between stimulation conditions by cluster analysis. Y-axis represents dissimilarity. .E. Compared to B-IVA, V-IVA resided in the same class (116.27 distance) and C-IVA was the next closest class (121.25 distance). B-Hep is the most dissimilar condition (135.24 distance). F. Compared to B-IVA, V-IVA resided in the same class (112.08 distance) and B-hep was the next closest class (125.78 distance). C-IVA is the most dissimilar condition (131.26 distance).

**Table 13. Comparison of each odor descriptor value between B-IVA and other stimulation conditions based on results of a t-test.**

| B-IVA vs C-IVA | | | B-IVA vs V-IVA | | | B-IVA vs B-Hep | | |
|---|---|---|---|---|---|---|---|---|
| ***O.D.*** | ***p-value*** | ***t-value*** | ***O.D.*** | ***p-value*** | ***t-value*** | ***O.D.*** | ***p-value*** | ***t-value*** |
| **sour** | 0.041 | 2.09 | **sickening** | 0.022 | 2.35 | **sickening** | 0.030 | -10.78 |
| **cheesy** | <0.001 | 4.66 | **rancid** | 0.020 | 2.39 | **aromatic** | <0.001 | -2.23 |
| **sour milk** | 0.002 | 3.23 | **putrid, foul, decayed** | 0.042 | 2.081 | **stale** | <0.001 | -2.73 |
| **fragrant** | 0.034 | 2.17 | **sewer odor** | 0.019 | 2.41 | **dirty linen-like** | 0.043 | -8.00 |
| **cinnamon** | 0.019 | 2.42 | **sour** | 0.018 | 2.44 | **rancid** | 0.025 | -7.87 |
| **household gas** | 0.039 | 2.11 | **sour milk** | 0.048 | 2.015 | **sweaty** | 0.008 | -8.56 |
| **peanut butter** | 0.008 | 2.72 | cedarwood -like | 0.035 | -2.16 | **putrid, foul, decayed** | <0.001 | -7.37 |
| **etherish, anaesthetic** | 0.030 | 2.23 | dry, powdery | 0.007 | 2.79 | **musty, earthy, moldy** | 0.006 | -5.20 |
| **buttery (fresh)** | 0.039 | 2.10 | dill-like | 0.014 | 2.54 | **like ammonia** | 0.035 | -5.73 |
| **soupy** | 0.006 | 2.82 | | | | **fecal (like manure)** | 0.001 | -7.19 |
| **dry, powdery** | 0.002 | 3.31 | | | | **heavy** | 0.014 | -2.066 |
| **creosote** | 0.018 | 2.43 | | | | **animal** | <0.001 | -6.69 |
| **caramel** | 0.046 | 2.03 | | | | **sewer odor** | 0.007 | -5.17 |
| **popcorn** | 0.013 | 2.56 | | | | **urine-like** | 0.002 | -5.19 |
| **spicy** | 0.019 | 2.40 | | | | **fermented (rotten) fruit** | <0.001 | -4.46 |
| | | | | | | **cheesy** | <0.001 | -4.39 |
| | | | | | | **light** | <0.001 | 2.30 |
| | | | | | | **sour milk** | <0.001 | -2.79 |
| | | | | | | **cadaverous, like dead animal** | 0.001 | -4.40 |
| | | | | | | **cat-urine-like** | 0.001 | -3.38 |
| | | | | | | **mouse-like** | <0.001 | -3.67 |
| | | | | | | **mushroom-like** | 0.034 | -2.17 |
| | | | | | | **yeasty** | <0.001 | -3.36 |
| | | | | | | **medicinal** | 0.002 | 2.16 |
| | | | | | | **sulphidic** | 0.002 | -2.76 |
| | | | | | | **fishy** | 0.001 | -3.30 |
| | | | | | | cool, cooling | <0.001 | 5.21 |
| | | | | | | fragrant | 0.007 | 7.36 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | cologne | 0.012 | 7.17 |
| | | | | | | fruity (citrus) | <0.001 | 2.86 |
| | | | | | | hay | <0.001 | -2.15 |
| | | | | | | fruity (other) | <0.001 | 2.53 |
| | | | | | | floral | 0.035 | 2.81 |

***O.D: Odor descriptor  two-tail t-test, DF=62***

Each stimulation condition is compared with B-IVA odor descriptor values from the odor quality rating task. This table only shows significantly different odor descriptors (remaining odor descriptors are n.s.). Odor quality descriptors which are also listed in UD (Table 11: the upper 25% of B-IVA odor quality descriptors) are indicated in bold.

### 2.5 Additional experiments find results similar to the first experiment.

To verify the effect of verbal cues on LD, I conducted a second experiment using the same experimental design with different groups. I hypothesized that for the second experiment if the verbal cue effect on LD is more intense than the random variation effect on LD, the first and second experiments would be correlated and if the verbal cue effect is not more intense, there would be no significant correlation. Sixteen participants in each group (total 16 X 3=48 participants) participated in the second experiment. Participants of Group 1 (assigned to B-IVA, B-Hep conditions in the first experiment, Table 8) were assigned to the C-IVA condition, participants of Group 2 (assigned to C-IVA condition in the first experiment, Table 8) were assigned to the V-IVA condition, and participants of Group 3 (assigned to V-IVA condition in the first experiment, Table 8) were assigned to the B-IVA and B-Hep conditions .

After verifying the statistical pattern of the second experiment's results, I compared differences of odor profiles between B-IVA, C-IVA, V-IVA, and B-Hep by separating UD and LD as in the first experiment. In UD, I found no significant differences among IVA conditions but observed significant differences compared to B-Hep (Figure 35 A). In LD, I found significant differences between IVA conditions and B-Hep condition (Figure 35 B). I also performed a multivariate analysis (principal component analysis), to observe spread patterns in the odor quality space. I found that dots of IVA conditions in UD were uniformly distributed, whereas B-Hep condition dots were scattered to the left side of IVA conditions dots (Figure 35 C). In LD, on the other hand, C-IVA dots were scattered to the right side of B-IVA and V-IVA (Figure 35 D). Cluster analysis showed an alteration in the distance between conditions. I found that UD showed more distant B-Hep with the IVA conditions compared to LD (Figure 35 E and F). Specifically, IVA conditions were clustered at a similar distance both in UD and LD, but B-Hep was clustered more distantly in UD compared to LD. Results of the second experiment suggest there were no significant differences between IVA conditions in UD, but a significantly reversed pattern was observed in LD as was found with the first experiment's results. Although there was less of an effect in the cluster analysis, most of the statistical patterns were the same as the first experiment.

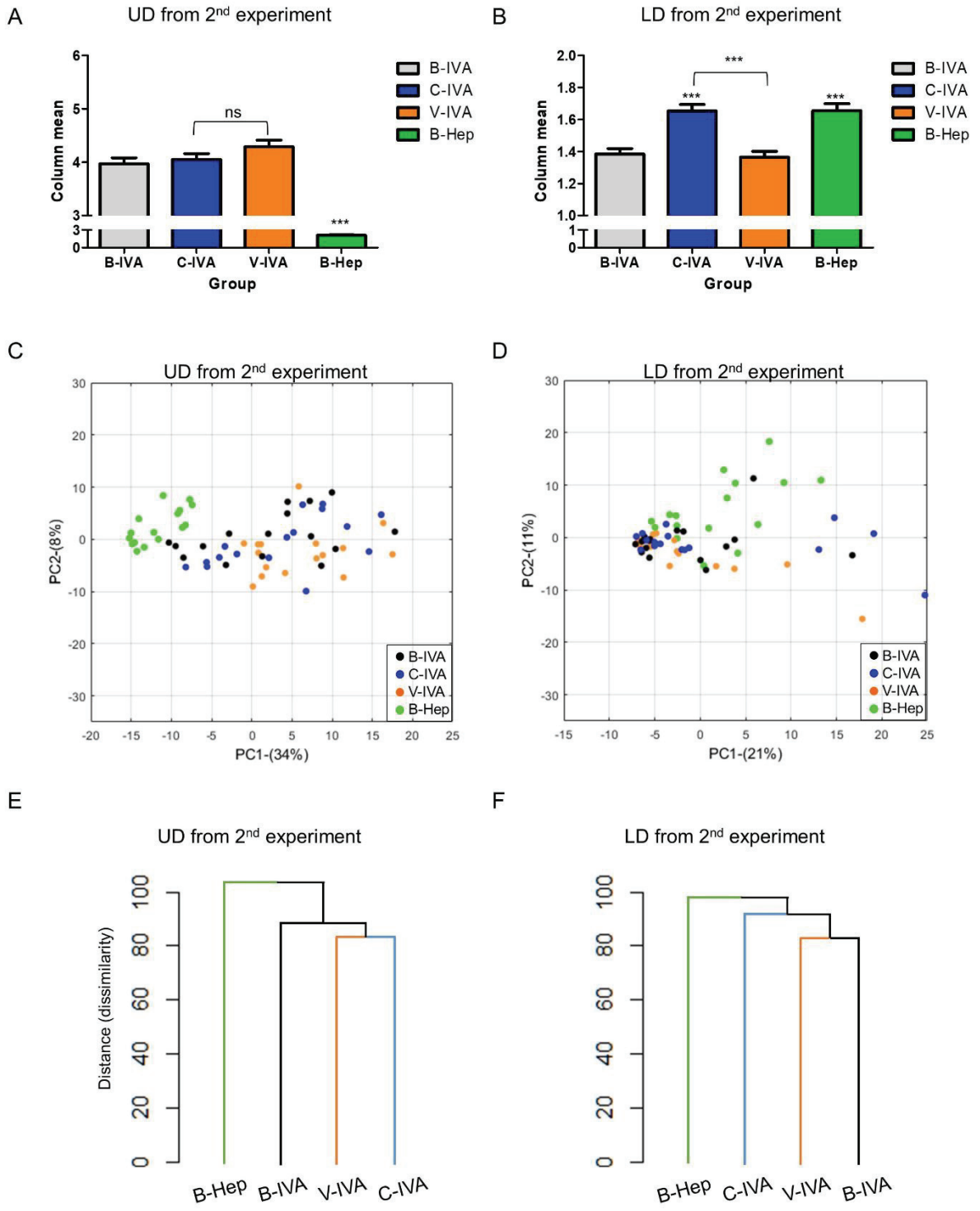



**Figure 35. Statistical patterns of odor descriptors from the second experiment.** A-B. Verification of differences in stimulation conditions. A. Based on results of a two-way ANOVA, B-Hep was significantly different from B-IVA but C-IVA and V-IVA had no differences. B. Based on results of a two-way ANOVA, C-IVA and B-Hep were significantly different from B-IVA. C-D. Odor quality space comprised PC1 (C: 34%, D: 21%) and PC2 (C: 8%, D: 11%). Each dot was projected from each participant's 37 descriptor values in C and 109 descriptor values in D. E-F. Verification of similarity between stimulation conditions by cluster analysis. Y-axis represents dissimilarity.

A correlation analysis was subsequently performed to verify precise similarities between the first and second experiments. I examined similarity between the average data of the randomly selected 16 participants in the first experiment and average data of the second experiment. As bias may affect correlational results, data from the first experiments were shuffled and used as a negative control. Due to the use of data from 16 randomly selected participants from the first experiment for correlation analysis, I first verified the number of sampling trials by evaluating the SEM (standard error of the mean) of the correlation coefficient (r-value). I found that trial 50 is the proper trial based on (Figure 36). I subsequently performed the correlation analysis and found a high correlation between both UD and LD (Figure 37). In UD, most of the trials had an r-value of 0.85 to 0.90 (Sum of trial=37). Moreover, LD also showed a high r-value of 0.65 to 0.75 (Sum of trial in 0.65 to 0.70 = 25, Sum of trial in 0.65 to 0.70 = 18). These findings suggest that the first and second experimental results were correlated for both UD and LD.

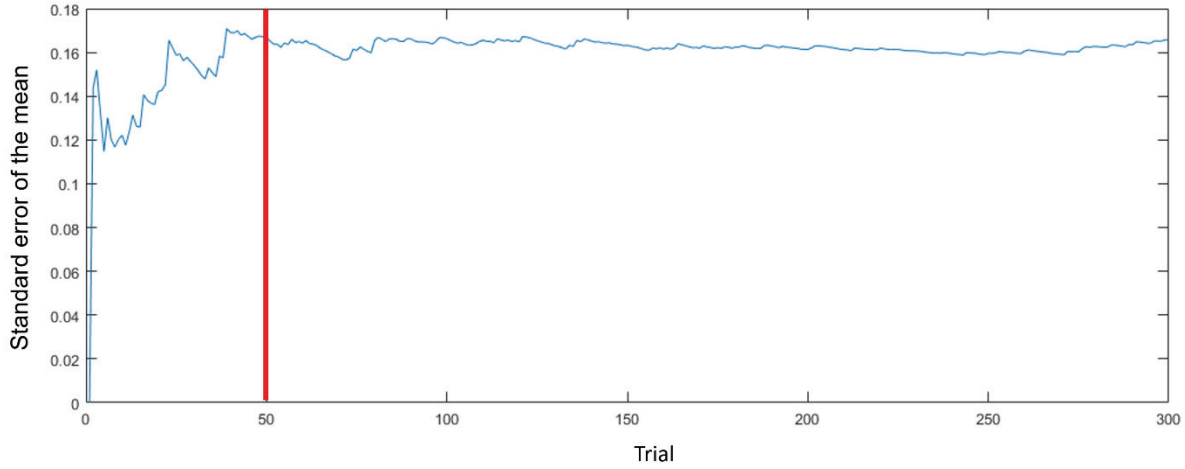



**Figure 36. Standard error of the mean (SEM) of the correlation coefficient based on trials.** X-axis is the trial. Y-axis is standard error of the mean. SEM has been shown to converge in over 50 tests (represented by the red line).

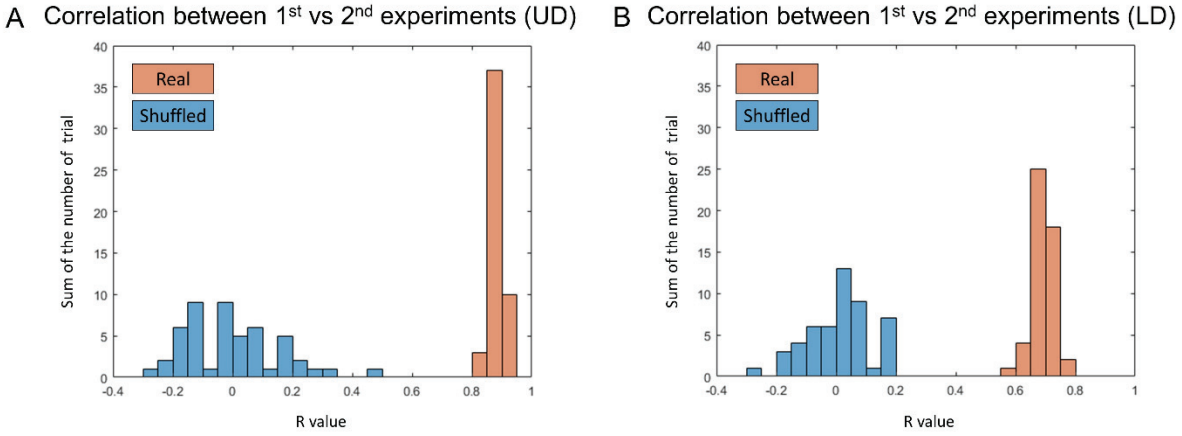



**Figure 37. Histogram of correlation analysis results between first and second experiments.** Orange is comparing real data and blue is comparing shuffled data of the first experiment. The x-axis is the correlation coefficient (r-value). Y-axis is the sum of the number of trials. A. Correlation results of first and second experiments in UD. Most of the trials showed an r-value of 0.85 to 0.90. B. Correlation results of first and second experiment data in LD. Most of the trials showed an r-value of 0.65 to 0.75.

## 3 Discussion

I found that the upper 25% of odor quality values (UD) were altered significantly less than the lower 75% of odor quality values (LD) in response to a verbal cue. These findings suggest that high ranked odor quality is less affected by verbal cues. I first verified the findings of previous studies [30,92] confirming that verbal cues induced alterations in odor quality responses (Figure 31) as was previously suggested [30,92]. Alterations in odor identification and emotional responses also support findings that verbal cues induce alterations in odor reactions (Figure 32, Table 9), and which is comparable with previous studies [32,47,84,93,137]. Notably, the effect of the verbal cue did not impact overall odor quality values, but only parts of odor quality values. As illustrated in Figure 33, distinctly different odors (B-Hep) had vastly different patterns in the upper 25% of odor quality descriptors of IVA (B-IVA). As Hep is a drastically different odor from IVA, the upper 25% of odor quality descriptors may be more related to specific IVA odor quality. Moreover, the current results showed that verbal cues induce much less alteration in high rated odor quality descriptors but strongly influence low rated odor quality descriptors (Figure 34). These results suggest that primary odor quality can be represented by the upper 25% of odor quality values, and verbal cues have only a weak influence on primary odor quality.

I first found that verbal cues exerted a weak influence on the high rated odor quality values (Figure 34). According to previous studies, training and experience may alter odor responses [80]. Moreover, cultural differences influence odor categorization [5,24] and individual differences in odor perception [51]. These findings suggest that rating odor quality is influenced by top-down modulation, including verbal cues which makes evaluating odor quality challenging. Similarly, the current findings suggest that verbal cues modulate odor quality responses, having more distance values (Figure 31B), and similar distance values compared to different odors (Figure 31D). However, I also found that these influences may not alter whole odor quality profiles and may primarily alter low ranked odor quality values (Figure 34). High rated descriptors were affected weakly, even when they were more related to verbal cues compared to low rated descriptors. Although high rated descriptors were also affected by cues (Table 13), these changes were not strongly affected when evaluating overall odor responses (Figure 34).

Weak alterations in UD may indicate that high rated odor quality represents the primary odor quality of

the targeted odorant, and these primary dimensions may be characterized by a subjective rating. From the molecular logic of smell, each chemical may have a specific odor quality as each chemical activates a specific OR repertoire set [35,62,83]. Subsequent studies suggest that the brain similarly categorizes chemical differences [44,99,120,141]. Moreover, despite having low performance on identifying and naming odor in humans [18,59], increasing evidence of high performance on odor discrimination ability in humans suggests that they may have sufficient ability to characterize each specific odor [16,73,77,133]. In agreement with previous studies, the current findings indicate that humans may perceive specific odor quality. According to my results, high rated descriptors were rarely affected by verbal cues (Figure 34A, C, E), although verbal cues can modulate parts of descriptors for high rated descriptors (Figure 31, and Table 13). The current studies suggest that high rated descriptors may represent the primary odor quality of the targeted odorant. Additionally, these findings imply that specific odor quality perception can be represented by a survey task rating odor descriptors.

However, I found that UD was also affected by verbal cues, though the influence was weak. Table 12 and Table 13 suggest that descriptors were altered by verbal cues, although they rated high enough to be categorized in the UD. One possible explanation for these alterations in UD is that the range of UD (upper 25%) was not perfect for representing specific odor quality. This is likely due to altered descriptors under cued conditions being rated lower compared to other UD descriptors (Table 12; C-IVA, V-IVA table). In contrast, for different odor conditions, entire odor descriptors were altered regardless of higher or lower ratings in UD (Table 12; B-Hep). These results suggest that higher rated UD descriptors may have a more appropriate representation of odor quality in experimental conditions, implying that inherent odor quality may not be perfectly represented by UD. An additional possible explanation is that altered descriptors may be related to verbal cues. Table 13 suggests that the 'cheese' cue condition (C-IVA) altered the 'cheesy' descriptors and other dairy descriptors (sour milk, sour). Moreover, the 'vomit' cue condition (V-IVA) altered 'sickening', 'rancid', and 'putrid, foul, decayed' which can be related to 'vomit'. These alterations occurred throughout the UD list, regardless of high or low rated descriptors. The current results suggest that some descriptors can be altered in response to cues. Therefore, a particular descriptor may be altered by proper cues that were highly relevant to the descriptor.

Some researchers have recently had success in predicting odor pleasantness and intensity of human

odor perception from chemical features and some semantic descriptors [67,69]. However, less predictive accuracy was shown on semantic descriptors, which may be a result of these descriptors having inherent limits [33,67,131]. Some research used alternative odor perception data such as odor similarity [113], which still has defects in representing the multidimensional axis of odor quality. Indeed, my method also has an issue with subjective rating but can, to some extent decrease individual alteration of odor quality. Results of the current study imply that people can measure the multidimensional axis of odor quality while reducing individual variations.

Notably, this study did not test all odors. Thus, I would not anticipate that all the odors have the same patterns as IVA in my study. However, it is clear from the current result that a verbal cue gives more alteration in LD than UD in the IVA odor profile (Figure 34). Previous studies suggest that primary odor quality can exist. Based on these findings, I hypothesized that primary odor quality may be less affected by top-down modulation such as verbal cues. In the current study, major alterations were shown in LD rather than UD which contains verbal cue related odor quality descriptors (Table 10), supporting my hypothesis. The reasons I chose IVA that it had already been tested in previous studies and was characterized as an ambiguous odor that can be influenced by verbal cues [29,47]. Moreover, verbal cues can modulate the cingulate cortex and OFC in the IVA condition and these findings suggest that IVA with verbal cues can be altered at the neurologic level [29]. Thus, IVA is one of the odors that have an easily alterable odor quality, and similar effects could be expected in less alterable odors. Another notable issue is the possibility that LD included more random variation than UD. I found LD was affected by verbal cues more than UD (Figure 34), but this influence could also be the result of random variation rather than verbal cues. Because LD includes descriptors less associated with an odor and may include more random variation than UD, these differences may be a contributing factor for the findings illustrated in Figure 34. However, the second experiment suggested that verbal cues had a notable impact on the LD (Table 10). Results of the correlation found an r-value of approximately 0.7 between the first and second experimental data in LD which represents a strong influence of verbal cues on LD descriptors. Indeed, if there is more random variation in LD than UD based on these results it could be concluded that verbal cues may be a major factor in the alteration of LD.

The current study suggests that people can extract essential odor quality descriptors which can more precisely represent an odor by sorting data. This was as indicated by the UD having better odor

quantification. The current study assessed differences in patterns between cue and no cue conditions in response to identical odor stimulation. I found that the higher-ranked odor quality (UD) may be less affected by top-down modulation (verbal cue) whereas the lower-ranked odor quality (LD) was notably affected compared to the high ranked odor quality. Although the numbers of odors tested in this study were limited, making it difficult to generalize for most odors, these findings provide measurement methods for a multidimensional axis of odor quality with increased accuracy.

# VIII. Conclusion

I studied odor categorization processing in the human brain by conducting experiments to examine temporal activation patterns. I established three studies to address this question. In study 1, I verified the possibility that direct olfactory processing signals can be measured at an early time point (within 200 ms). I initially found that ERP signals observed during odor conditions occur within 200 ms (Table 3). To further confirm these results, I designed an experimental procedure to examine odor habituation (Figure 15). Participants were offered odors for 30 s to desensitization OSNs in the OE. For the behavioral test, I chose heptanol and 2-acetylpyrazine, which are perceptually and structurally different odors, and confirmed that the intensity of odor was significantly decreased under the “same” condition, not but under “different” and “none” conditions (Figure 16). These results suggest that odor habituation occurred mainly when the same odors were repeated, whereas cross adaptation caused by the two odors was difficult to detect. These findings are in line with previous odor habituation studies [96,97]. I found that ERPs differed within 200 ms depending on the conditions (Table 4 and Table 5). In several channels, the amplitude and latency of the NP or PP differed within 200 ms under the “same” condition compared with other conditions. To examine whether these ERP changes were related to the behavior, I performed a correlational analysis comparing the ERPs and behavioral results. I found a significant correlation between the behavior and ERPs within 200 ms, primarily in channels located in the right temporal and parietal lobe areas (Figure 18 and Figure 19), implying that the information for odor habituation may be processed centrally at early time points.

In a second experiment, I examined temporal patterns during olfaction using similar and dissimilar odors. I found that similar odors induced similar theta activities within 100 ms. Specifically, brain activity was measured following stimulation with two odors perceived as similar (i.e., AP and TP). Odorants were classified by ERSP and represented similar temporal and spatial patterns between similar odors especially in theta (Figure 25 and Figure 27). Looking closer at the timeframe of these responses, spatial patterns of AP and TP clustered in the same class within 100 ms and 150-200 ms (Figure 25). Moreover, multivariate patterns of theta and gamma also indicated that AP and TP induced similar ERSP patterns within 50—200 ms and 350-400 ms (Figure 27). These results suggest that odor

information encoding may occur within 100 ms after odor stimulation and theta waves may be heavily involved. Additionally, categorizing events can be represented within specific periods rather than overall periods.

In a third and final study, I characterized odor similarity considering the multidimensional axis of odor object quality. I found that the upper 25% of odor quality values (UD) were altered significantly less than the lower 75% of odor quality values (LD) in response to a verbal cue. These findings suggest that high ranked odor quality is less affected by verbal cues. I first verified the findings of previous studies [30,92] that report verbal cues can induce alterations in odor responses. I confirmed previous findings that verbal cues indeed induce alterations in odor quality responses (Figure 31) as previously suggested [30,92]. Alterations in odor identification and emotional responses also support findings that verbal cues induced alterations of odor reactions (Figure 32, Table 9) in agreement with previous studies [32,47,84,93,137]. Notably, the effect of a verbal cue did not impact overall odor quality values, rather it impacted parts of odor quality values. As shown in Figure 33, odors that were drastically different (B-Hep) had remarkably different patterns in the upper 25% of odor quality descriptors of IVA (B-IVA). Because Hep is a totally different odor from IVA, the upper 25% of odor quality descriptors may be more closely related to IVA specific odor quality. Moreover, the current studies found that verbal cues induce much less alteration in high rated odor quality descriptors but strongly influence low rated odor quality descriptors (Figure 34). These results suggest that primary odor quality can be represented by the upper 25% of odor quality values, and verbal cues may have a weak influence on primary odor quality.

One of the limitations of the current study was a lack of evidence regarding brain location information. Because the current study used EEG which has low spatial resolution it is difficult to determine which brain areas were activated. This means that although I found features of odor categorization by EEG, signals may be derived from top-down processes or from brain areas that are not related. The range of the time window for theta waves should be considered as well. Because theta frequency is 4-9 Hz, I set a 50 ms range for the time window. Although I found consistent results from theta, further study is needed to draw more concrete conclusions. The odor delivery method also should be considered as the effect of variations of the olfactometer that was used are unknown. Stimulation onset can be measured, during which I found variation was less than 1 ms. However, in experimental conditions, each participant has individually different respiration speed, therefore this factor could influence

stimulus time and alter results between participants.

Through these studies, I found that theta and gamma waves display important temporal patterns in the early stages of odor classification. In particular, between 50 and 100 ms during the active time zone, the primary olfactory cortex first starts to be activated during olfactory signal processing. This means that odor classification can be performed before interacting with cognitive functions such as memory during the olfactory process, and can serve as an objective odor classification index based on the activation pattern at this time point. Therefore, I suggest that people perceive odor objectively at least in the 50-100 ms period after odor recognition. Moreover, this objective feature may be sustained even at the time of behavioral output. Although verbal cues altered odor quality perception, differences in odor can be sorted. The current studies may help in standardizing odor measurement in academic and industrial fields.

# IX. Nomenclature

| | |
|---|---|
| ANOVA | Analysis of variance |
| AP | 2-acetlypyrazine |
| EEG | Electroencephalography |
| ERP | Event related potential |
| ERSP | Event related spectral perturbation |
| fMRI | Functional magnetic resonance imaging |
| Hep | Heptanol |
| iEEG | Intracranial EEG |
| IVA | Isovaleric acid |
| MEG | Magnetoencephalography |
| OB | Olfactory bulb |
| OE | Olfactory epithelium |
| OFC | Orbitofrontal cortex |
| OR | Olfactory receptor |
| OSN | Olfactory sensory neurons |
| PC | Piriform cortex |
| PCA | Principal component analysis |

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# 요 약 문


일상 속에서 우리는 대상을 분별하고 또 인식하면서 살아간다. 하지만 우리는 과연 대상을 얼마나 객관적으로 인식하고 분별할 수 있는가? 더불어 얼마나 정확한 정보를 다른 사람에게 전달하고 있을까? 이 논문은 이러한 질문으로부터 출발하였고, 다른 감각에 비해 주관성이 강한 후각 정보를 분석하여 위와 같은 질문을 풀고자 하였다. 따라서 나는 사람이 냄새 정보를 어떻게 처리하는 지 그 기작을 연구하는 것에 집중하였고, 뇌신호를 이용하여 본 연구를 진행하였다.

나는 특히 뇌신호의 시간적 패턴 변화에 집중을 하였다. 우리의 신경은 시공간의 방법을 모두 이용하여 정보를 coding한다. 더불어 우리의 뇌는 어떤 부위가 언제 활성화 되는가에 따라 여러가지 다른 기능들을 수행한다. 하지만 사람 연구의 경우, 비교적 공간 기반의 뇌신호 연구 중심으로 진행되었고, 따라서 뇌가 어떻게 냄새 정보 처리 시 기능을 수행하는지 충분한 정보를 제공하지 못하였다. 이에 시간 분해능이 좋은 EEG를 사용하여 연구를 진행하였다.

첫번째 연구는 EEG 신호로 후각 신호를 측정 할 수 있는지 확인하였다. 왜냐하면 후각 신호는 뇌의 심부층에서부터 처리되기에 EEG신호가 약해질 수 있고, 이전 in vitro 연구들에서는 후각 정보가 200ms 이후에 전달된다고 알려졌기 때문이다. 비록 최근 MEG 또는 행동 연구에서 후각 신호가 200ms 이전에 처리될 수 있다고 알려졌으나, EEG 신호로 후각 신호를 직접 측정할 수 있는지는 알려지지 않았다. 확인한 결과 후각 특이적인 신호가 200ms 이전에 측정되었고 (약 110ms), 후각 자극이 변화할 시 이 신호가 변화하는 것이 확인되었다.

두번째 연구에서는 첫번째 연구를 기반으로 냄새 분별 과정을 시간적 측면에서 접근하여 세부적으로 확인하였다. 이를 위해 유사한 향 두개와 전혀 다른 향 하나를 선별하여 이들의 EEG 신호의 유사성을 확인하였다. 확인한 결과 유사한 향들은 theta에서 50-100ms, 150-200ms, 그리고 350-400ms의 시간대에서 유사한 패턴을 보임이 확인되었다. Gamma파의 경우는 100-150ms 그리고 상대적으로 늦은 시간대인 350-400ms에서 유사한 냄새 분별 패턴이 확인되었다. 또한 이러한 결과는 위치 기반 분석을 통해 후각 관련 뇌영역과 관련이 있는 것으로 나타났다. 이 연구를 통해 냄새 구별은 특히 특정 시간 범위에서 후각 관련 뇌영역 (PC, OFC 등)들을 통해 처리된다고 확인되었다.

세번째 연구에서는 행동 수준에서의 냄새질 (odor object quality)을 특성화 하는 것에 집중하였다. 이전 연구에서 냄새 구별 과정이 뇌파로 표현 될 수 있음을 이미 확인했지만, 본 논문의 목적은 냄새 정보 처리에 대한 이해를 더하는 것이기에 행동 수준에서의 냄새 연구 역시 진행하였다. 본 논문의 연구1, 2에서도 제시된 바와 같이 최근 생리학 및 행동 연구에서는 냄새 품질을 특성화 할 수 있는 몇가지 특징들을 밝혀냈다. 하지만 냄새질의 다차원 축을 측정하는 방법은 아직까지 어렵다. 더욱이 개인의 경험에 의해 냄새질이 바뀔 수 있다는 추가적인 문제도 있다. 따라서 verbal cue를 사용하여 변화된 냄새 반응을 정량화하고 특성화하여 냄새 간 차이를 알 수 있는 구분 기준을 제안하려고 하였다. 본 연구를 통해 높은 점수 (상위 25 %)의 냄새 표현은 경험에 따라 크게 변하지 않는 것으로 나타났고, 따라서 이 결과를 통해 보다 정확한 냄새 정량화가 가능함을 시사하였다.

이 연구들을 통해서 나는 theta 및 gamma가 냄새 분별 초창기에 중요한 주파수 임을 알 수 있었다. 특히나 주로 활성화된 시간대가 50-100ms 사이는 후각 신호 처리 과정 중 일차 후각 피질이 처음으로 활성화 되기 시작하는 시간대이다. 이는 냄새 분별이 후각 처리 과정 중 기억과 같은 인지 작용과 상호작용 하기 이전부터 명확하게 이루어 질 수 있다는 점을 의미하며, 이 시간대의 활성화 패턴을 통해 객관적인 냄새 분별 지표가 될 수 있음을 의미한다. 따라서 나는 최소한 냄새 인식 후 50-100ms 사이 시간대에서는 사람들이 냄새를 객관적으로 인식하고 있다고 제안한다. 또한 아직 증거는 적지만 이러한 뇌에서의 냄새 구별 기작이 행동적으로도 표출될 수 있음을 제안한다.

핵심어: 냄새질 (Odor object quality), 냄새질 특성화 (characterizing odor quality), 후각 처리 (olfactory processing), 시간 패턴 코딩 (temporal pattern coding), 사람 뇌 (human brain)