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Expanded olfactometer for measuring reaction time to a target odor during background odor presentation

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Abstract

Current olfactometers can insert a target odor into the flow of odorless air as a pulse (i.e., replace odorless air with target odor for a very short time), but no previously designed olfactometer can insert a pulse of target odor into a flow of background odor (i.e., replace background odor with target odor for a very short time). To measure reaction time to a target odor during presentation of a background odor, we developed an expanded olfactometer by adding an attachment to an existing olfactometer. We conducted three experiments to evaluate the performance of the expanded olfactometer. Additionally, four volunteers participated in trial measurement of reaction time for detection of the target odor under background odor and odorless air conditions using the expanded olfactometer. We did not observe a significant difference in gas onset time or rise time of the target odor between background and odorless air conditions. Additionally, the gas onset time and rise time of the target odor were on the order of milliseconds, whereas the gas onset time and rise time of the background odor were on the order of seconds. The reaction time was marginally significantly longer under the background odor condition than the odorless air condition. We did not observe a

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significant difference in gas onset time or rise time of the target odor between the existing olfactometer and our expanded olfactometer. We succeeded in developing an attachment capable of inserting a target odor into a flow of background odor. Our results revealed that performance related to the presentation of the target odor was comparable between the existing and expanded olfactometers. To more rigorously examine the effect of background odor on detection speed of target odor, we intend to increase the number of participants in the near future.

Keywords: Neuroscience, Psychology

1. Introduction

When measuring reaction time to a target odor, gaseous odor must be strictly controlled on the timescale of milliseconds. Additionally, olfaction must be stimulated without causing other stimuli such as tactile, pain, cold, or warm sensations. Evans et al. (1993) proposed requirements for measuring olfactory evoked potential with high accuracy. The first requirement is the ability to insert an odor into a flow of odorless air as a pulse. If an olfactory stimulus is blown into the nasal cavity, a tactile sensation is also induced as the pressure changes. Second, the olfactory stimulus must be presented in a rectangular wave-form. More specifically, the time to reach 70% of maximum concentration must be within 50 ms from stimulus onset. Third, odorless air must be presented in the nasal cavity throughout the measurement. However, in order to prevent drying of the nasal cavity, the humidity of odorless air must be greater more than 50%, and its temperature must be similar to body temperature (35−37 °C). Kobal and colleagues (Kobal, 1985; Kobal and Hummel, 1988) succeeded in developing an olfactometer that satisfies all these criteria.

In conventional measurements of reaction time (Boesveldt et al., 2010; Jacob and Wang, 2006; La Buissonnière-Ariza et al., 2013; Olofsson et al., 2013) to a target odor, the odor is inserted as a pulse into a flow of odorless air. However, in everyday life, we often unexpectedly notice another odor during exposure to an odor of interest, e.g., city gas odor appears when we are cooking, or cigarette odor appears when we are sitting at the dinner table. Thus, humans are constantly exposed to chemically complex stimuli (Sinding et al., 2013). There are few opportunities to smell monomolecular odors in everyday life, and olfactory function is primarily used to process complex mixtures of odorants present in the environment (Thomas-Danguin et al., 2014). The olfactory system may recognize complex mixtures of odorants as single entities (configural perception) or discriminate a specific odor from complex mixtures (elemental perception) (Barkat et al., 2012; Coureaud et al., 2008). When a given odor (target odor) is discriminated from another aroma (background odor), the olfactory system needs not only to detect the individual features of each odor, but also to decide whether the source of each feature is the target or background

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odors (Wilson and Stevenson, 2003). Accordingly, it is possible that the time required for olfactory information processing of the same target odor could differ between conditions in which a background odor is present or absent.

The time required for information processing is commonly evaluated using the reaction time paradigm (Burke et al., 2017). In this study, we developed an olfactometer for measuring reaction time to a target odor during presentation of a background odor. More specifically, we added an attachment capable of inserting a pulse of target odor into a flow of background odor (i.e., replacing odorless air with the target odor for a very short time) to the olfactometer developed by Kobal and colleagues (Kobal, 1985; Kobal and Hummel, 1988). To evaluate the performance of the olfactometer with the attachment (hereafter, referred to as the expanded olfactometer), we conducted three experiments using a high-speed ultrasonic gas sensor (Toda and Kobayakawa, 2008; Toda et al., 2005). In Experiment 1, we compared the gas onset time and rise time of the target odor between a condition in which the background odor was present (background odor condition) and one in which it was absent (odorless air condition). In Experiment 2, we compared the gas onset time and rise time of the target odor between the existing olfactometer and our expanded olfactometer. In Experiment 3, we measured the gas onset time and rise time of the background odor. Gas onset time and rise time were calculated based on the output from the high-speed ultrasonic gas sensor, converted into voltage. Additionally, as an example of a psychophysical experiment using the expanded olfactometer, we compared the reaction time for detection of the target odor under the background odor and odorless air conditions.

2. Instrumentation

2.1. Existing olfactometer

A schematic of the olfactometer (Olfactometer OM4; Burghart Instruments, Wedel, Germany) developed by Kobal and colleagues (Kobal, 1985; Kobal and Hummel, 1988) is shown in Fig. 1(a). This olfactometer consists of a line through which odorless air flows, a line through which odor (odorized nitrogen) flows, and a line for exhausting unpresented gases. The odor consists of nitrogen gas odorized by passing through an absorbent cotton bearing the odorant. By switching between odorless air and odor lines using a three-way solenoid valve, the odor was exhausted via a suction pump during the presentation of odorless air; likewise, odorless air was exhausted during the presentation of the odor.

A gas wash bottle (model number PFA 100; As One Corporation, Osaka) made of fluorine resin was placed at the inlet as a heating module for gaseous odor. This module was a component originally incorporated in the existing olfactometer ("Olfactometer OM4") developed by Kobal and colleagues (Kobal, 1985; Kobal and

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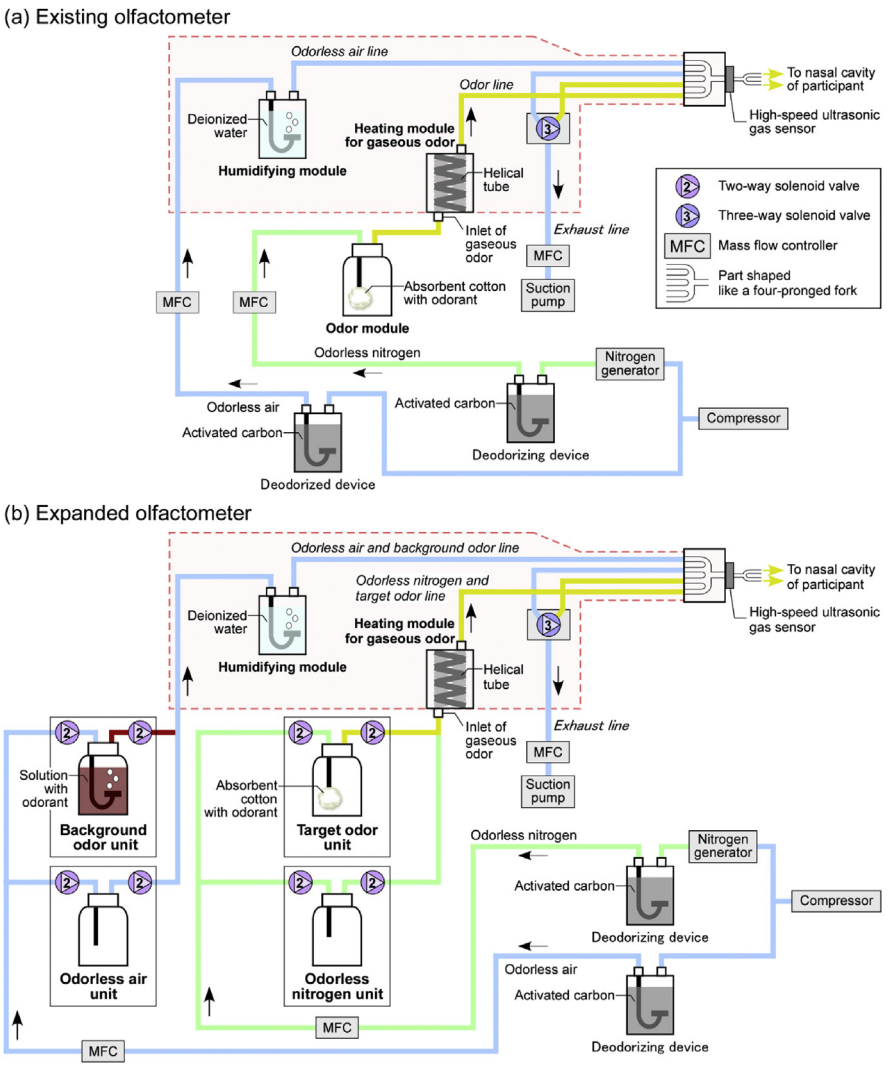

Fig. 1. Outlines of existing and expanded olfactometers. The existing olfactometer (a) consisted of an odorless air line, an odor line, and an exhaust line. In the expanded olfactometer (b), two units (odorless air and background odor) were arranged in parallel in the middle of the odorless air line of the existing olfactometer. Additionally, two units (odorless nitrogen and target odor) were arranged in parallel in the middle of the odor line of the existing olfactometer. The part surrounded by the dotted line was heated by circulating warm water around the lines.

Hummel, 1988). In many cases (e.g., Kettenmann et al., 1997), the inlet of the heating module for gaseous odor is connected to a high-pressure gas cylinder containing an odorized gas. However, the type of gaseous odors supplied in high-pressure cylinder format is limited. Therefore, we connected a gas wash bottle containing an absorbent cotton bearing the odorant (i.e., odor module) instead of a high-pressure gas cylinder. The pressurized odorless nitrogen passed through the gas wash bottle, thereby generating an odor. The odor module was not heated, but warm water circulated around the odor line from heating module for gaseous odor to near the outlet of olfactometer.

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At the outlet of the olfactometer, we attached a high-speed ultrasonic gas sensor (Toda and Kobayakawa, 2008; Toda et al., 2005) and a forked thin tube made of polypropylene and Teflon. The participant inserted this tube approximately 1 cm into both nasal cavities. To avoid pressure changes and temperature changes in the nasal cavity, odorless air was always presented through the tube into the nasal cavity, and odorized nitrogen was inserted into the flow of odorless air as a pulse. More specifically, by controlling the three-way solenoid valve with output from a digital input/output board on a personal computer (PC), gas presented to the participant was switched from odorless air to odor, and then back to odorless air. To perform real-time monitoring of gas presented to the participant, a high-speed ultrasonic gas sensor (Toda and Kobayakawa, 2008; Toda et al., 2005) was placed at the outlet of the olfactometer. The high-speed ultrasonic gas sensor converts the molecular weight of gas into a voltage value. This sensor can successfully detect gas exchange between air (average molecular weight 28.8) and nitrogen (molecular weight 28) with a signal-to-noise ratio that is larger than 42 dB, and a temporal resolution of detection is less than 1 ms (Toda et al., 2005). Additionally, humidified air can be discriminated from non-humidified air based on the output from the high-speed ultrasonic gas sensor. However, the possibility of discrimination between humidified odorless air and humidified odorized air depends on the saturated vapor pressure of a given odorant. Changes in voltage values, based on the outputs from the PC that controlled the three-way solenoid valve and the high-speed ultrasonic gas sensor, were processed by an analog-to-digital conversion circuit (PowerLab; ADInstruments, Bella Vista, Australia), and the digitized value was recorded.

The flow rates of odorless air and odorless nitrogen were controlled by mass flow controllers. To supply odorless air and odorless nitrogen to the olfactometer, atmosphere was taken into the olfactometer by a compressor, and then the odorants contained in the atmosphere were removed by passing the air through a deodorizing device, which was a gas wash bottle made of stainless steel (diameter 73 mm $\times$height 170 mm) with 250 g of granular activated carbon (model number 4GG for vapor phase, pellet 4mm; As One Corporation, Osaka). To humidify the odorless air, it was passed through a gas wash bottle containing deionized water. To keep the odorless air and odor warm, water of approximately 40 °C was circulated around the Teflon tube, which was the path for both the odorless air and odor.

2.2. Expanded olfactometer

2.2.1. Outline

To present the background odor, an attachment was added to the existing olfactometer, as shown in Fig. 1(b). Two units (odorless air unit and background odor unit) were arranged in parallel in the middle of the odorless air line of the existing olfactometer. Similarly, two units (odorless nitrogen unit and target odor unit) were

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arranged in parallel in the middle of the odor line of the existing olfactometer. Teflon tubes (model number F-8011, inner diameter 4.35 mm × outer diameter 6.35 mm; Flon Industry, Tokyo) and three types of Teflon connectors were used to connect the parts. An elbow Teflon connector (model number 30-6MCT 4-C; Flowell Corporation, Yokohama, Japan) or a T Teflon connector (model number 30-6MBT 4-C; Flowell Corporation, Yokohama, Japan) was attached to the two-way solenoid valve (model number MYB2-6-DC24V; CKD Corporation, Aichi) of each unit. To connect two Teflon tubes, an elbow Teflon connector (model number 30-6UE-C; Flowell Corporation, Yokohama, Japan) was used.

2.2.2. *Electronic circuit for driving the two-way solenoid valve*

A diagram of the electronic circuit for driving the two-way solenoid valve of each unit is shown in Fig. 2. The two-way solenoid valves of each unit were controlled by output from the PC via a microprocessor (model number Arduino Uno Rev3; Arduino Srl, Ivrea, Italy) and semiconductor relays ("PhotoMOS® relay", model number AQW 212; Panasonic Corporation, Kadoma, Japan). The microprocessor was

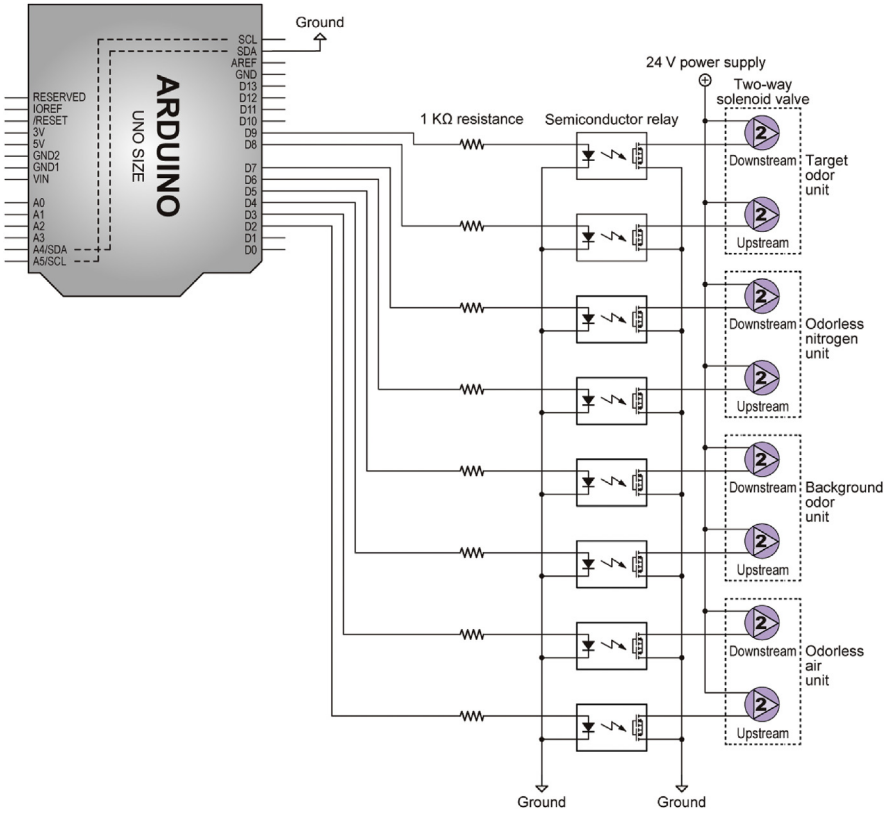

Fig. 2. Electronic circuit diagram for driving two-way solenoid valves of each unit. The device attached to the existing olfactometer consisted of four units. An electronic circuit diagram for driving eight two-way solenoid valves (the upstream and downstream solenoid valves per unit) is shown.

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connected to the PC via a universal serial bus (USB) cable. The three-way solenoid valve of existing olfactometer was also controlled by output from the same PC. A semiconductor relay was connected to each channel (D2−D9) of the microprocessor. A voltage signal of 5 V, which was outputted from the microprocessor, controlled application of voltage of 24 V to the two-way solenoid valve via the semiconductor relay.

The two-way solenoid valves of the odorless air and background odor units were open and closed at steady state, respectively. Similarly, the two-way solenoid valves of the odorless nitrogen and target odor units were open and closed at steady state, respectively.

2.2.3. Background odor and odorless air conditions

Under the background odor and odorless air conditions, odorless air was presented constantly in the nasal cavity of the participant by switching the three-way solenoid valve. Odorless nitrogen was exhausted via a suction pump, without being presented in the nasal cavity of the participant, by switching the three-way solenoid valve. When target odor was presented, odorless nitrogen and target odor were switched using the two-way solenoid valves of each unit.

Under the background odor condition, odorless air and background odor were switched using the two-way solenoid valve of each unit. To insert a target odor into the flow of background odor as a pulse, the background and target odors were switched using the three-way solenoid valve. On the other hand, under the odorless air condition, odorless air and background odor were not switched. To insert a target odor into the flow of odorless air as a pulse, odorless air and target odor were switched using the three-way solenoid valve. Incidentally, results of Experiment 3 (described below) showed that the expanded olfactometer required approximately 4 s for the background odor, which was exchanged with odorless air by switching the two-way solenoid valves of each unit, to reach equilibrium in the line of odorless air and background odor. The time required for odorless air, odorless nitrogen, and the target odor to reach equilibrium in each line was also comparable to the time required for the background odor.

3. Experimental

3.1. Experiment 1: Comparison of gas onset time and rise time of target odor between background odor and odorless air conditions

3.1.1. Timing of solenoid valve control

The timeline of the solenoid valve control in each trial is shown in Fig. 3.

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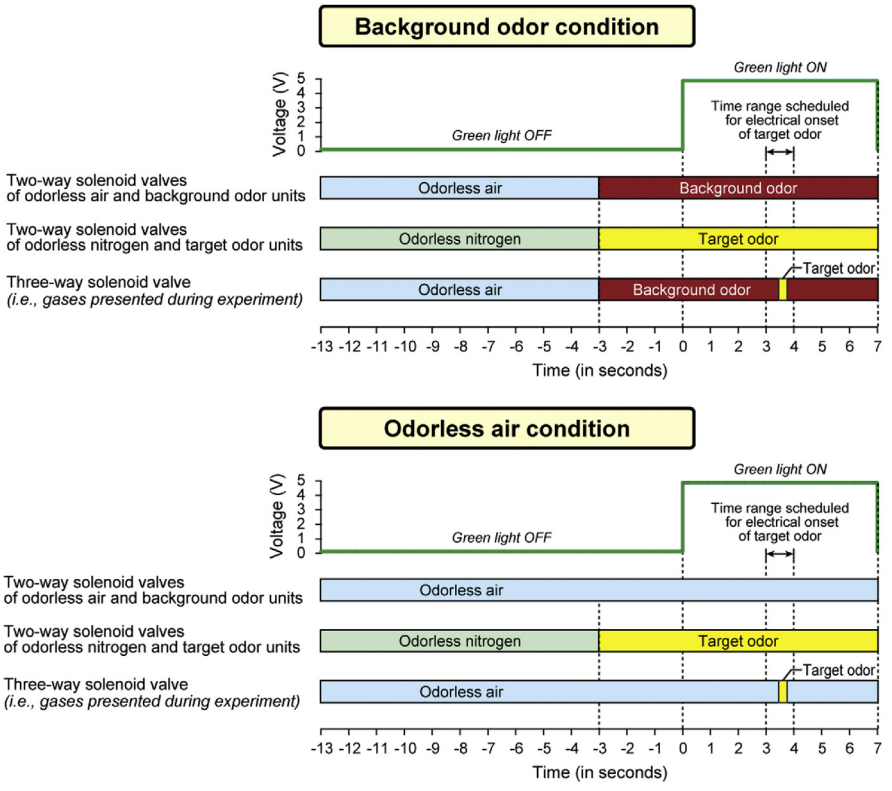

Fig. 3. Timeline of solenoid valve control in each trial. Timeline The timeline of the three-way valve represents the gases presented from the outlet of the olfactometer under each condition. The presentation time of target odor was 0.3 s (300 ms). The presentation time of background odor was 10 s (actual time was 9.7 s; 0.3 s out of 10 s was spent to present the target odor) from 3 s before the green light was turned on to 7 s after the green light was turned on. The electrical onsets of the target odor were randomized among trials.

a. Background odor condition

During the measurement, odorless air flowed constantly into the participant's nasal cavity. Switching from odorless air to background odor was performed 3 s before the green light was turned on, and background odor began to flow into the participant's nasal cavity. At the same time, switching from odorless nitrogen to target odor was performed. Switching from background odor to target odor was conducted within 3−4 s after the green light was turned on (i.e., corresponding to electrical onset), and the target odor was presented in the participant's nasal cavity. Electrical onsets were randomized among trials. After the target odor was presented for 300 ms, switching from target odor to background odor was performed. Switching from background odor to odorless air occurred 7 s after the green light was turned on (i.e., when the green light was turned off), and odorless air began to flow again into the participant's nasal cavity. At the same time, switching from target odor to odorless nitrogen was performed.

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b. Odorless air condition

The procedure was the same as for the background odor condition, except that switching between odorless air and background odor was not performed.

3.1.2. Procedure

We poured 50 mL of deionized water into the gas wash bottle of the background odor unit. The tip of the Teflon tube inserted into the gas wash bottle of the target odor unit was wrapped with a 2.5 cm × 2.5 cm absorbent cotton, which was fixed to the tube with a thin wire. We dropped 1 mL of propylene glycol (special grade reagent; Wako Pure Chemical Industries, Osaka, Japan) onto the absorbent cotton using a microsyringe.

The flow rate of odorless air and background odor was 7.2 L per minute, the flow rate of odorless nitrogen and target odor was 5.0 L per minute, the flow rate of exhaust was 5.8 L per minute, the presentation time of background odor was 10 s (actual time was 9.7 s; 0.3 s out of 10 s was spent to present the target odor), the presentation time of target odor was 0.3 s (300 ms), and the presentation interval between target odors was approximately 20 s. The green light was turned on for 7 s per trial. Measurements under background odor and odorless air conditions were performed alternately for 20 trials each. The aforementioned flow rates of gases and exhaust were the values displayed on the control unit of olfactometer. Before starting the experiment, we adjusted the flow rates of gases and exhaust by placing a U-tube manometer at the outlet of the olfactometer. More specifically, the flow rate of odorless air and background odor displayed on the control unit was set to 7.2 L per minute. Subsequently, the line of odorless nitrogen and target odor was closed by shutting off the power of mass flow controller, but the exhaust line was opened. The pressure applied to the exhaust line was adjusted to 20 cmH_2O (= 1.96 kPa) higher than the pressure applied to the line of odorless air and background odor, by operating the control unit. Finally, the line of odorless air and background odor was closed by shutting off the power to the mass flow controller, whereas the line of odorless nitrogen and target odor was opened. The exhaust line remained open. The pressure applied to the exhaust line was adjusted to 20 cmH_2O (= 1.96 kPa) higher than the pressure applied to the line of odorless nitrogen and target odor, by operating the control unit. When we adjusted the flow rates of gases and exhaust using the U-tube manometer, the flow rate of odorless air and background odor was almost equivalent to the flow rate of odorless nitrogen and target odor. Additionally, by smelling the gases at the outlet of olfactometer every time the flow rates were adjusted, two experimenters confirmed that there was no perceptual difference in the flow rates among the gases.

For the measurement of reaction time (described below), the green light was used as a fixation point and warning light for odor presentation. Power to the green light was

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controlled by a voltage of 5 V outputted from the PC. In order to control the three-way solenoid valve, another voltage of 5 V was outputted from the PC corresponding to illuminating the green light. Changes in the illuminating and extinguishing the green light, the voltage value based on the output from the PC to the three-way solenoid valve, and the output from the high-speed ultrasonic gas sensor were recorded at a sampling rate of 1,000 Hz. In the performance evaluation, it was possible to discriminate between odorless air and the target odor (nitrogen containing molecules of propylene glycol), and between the background odor (non-odorized air) and target odor on the basis of the output from the high-speed ultrasonic sensor. On the other hand, in Experiment 1, because the odorless air and background odor were humidified, it was impossible to discriminate between these gases on this basis.

3.1.3. Analysis

We defined the gas onset time as the latency from electrical onset (the time point at which the signal controlling the solenoid valve, which was used to switch between gases, was output by the PC) to gas change onset (Toda et al., 2005). To calculate the gas onset time for each trial, we used 20,000 points of voltage value data (20 s × sampling rate 1,000 Hz) recorded over 20 s, starting with the illumination of the green light. After we identified the minimum and maximum values of these data, we divided the interval between the minimum and maximum values into 20 bins (i.e., every 5%). Nineteen voltage ranges were arranged by moving the voltage range of 10% from the minimum value to the maximum value in increments of 5% (i.e., 0−10%, 5−15%, 10−20%, ….., and 90−100%). Because we identified a voltage range including more than 95% (i.e., 19,000 out of 20,000 points) of voltage data in all trials, we considered that the data included in the identified voltage range corresponded to the baseline voltage value; accordingly, the average and standard deviation (SD) of these data were calculated. We defined the time point at which the voltage value was above the average +5 SD as 'gas exchange onset'. The gas onset time was calculated by subtracting the electrical onset from the gas exchange onset.

We defined the rise time as the latency from gas exchange onset to arrival at 70% of the maximum concentration. To calculate the rise time of each trial, we regarded the average value of the baseline as 0% and the minimum voltage (i.e., maximum concentration) as 100%, and then identified the time point at which the voltage value was over 70% of the minimum voltage. The rise time was calculated by subtracting the gas exchange onset from the time of arrival at 70% of the minimum voltage.

To determine whether gas onset time and rise time of the target odor differed between the background odor and odorless air conditions, we performed the unpaired *t*-test for each parameter. Throughout this study, we used IBM SPSS Statistics 23 (IBM Japan, Tokyo) for statistical analysis, and p values less than 0.05 were

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considered statistically significant. No correction was performed to adjust the significance level.

3.1.4. Results

An example of real-time monitoring of a target odor with a high-speed ultrasonic gas sensor under the background odor and odorless air conditions is shown in Fig. 4. In this experiment, both the odorless air and background odor (non-odorized air) were humidified; thus, it was impossible to discriminate between them based on the output from the high-speed ultrasonic sensor. Therefore, the output from the high-speed ultrasonic gas sensor recorded during real-time monitoring of the target odor did not differ between the background odor and odorless air conditions.

The gas onset times (means ±SD) of the target odor were 60.65 ± 0.67 ms under the background odor condition and 60.30 ± 0.73 ms under the odorless air condition. The rise times (means ±SD) of the target odor were 51.70 ± 3.79 ms under the background odor condition and 52.25 ± 3.67 ms under the odorless air condition. For gas onset time as well as rise time, there were no significant differences between conditions (unpaired *t*-test). These results demonstrate that performance related to the presentation of the target odor was equivalent between the background odor and odorless air conditions.

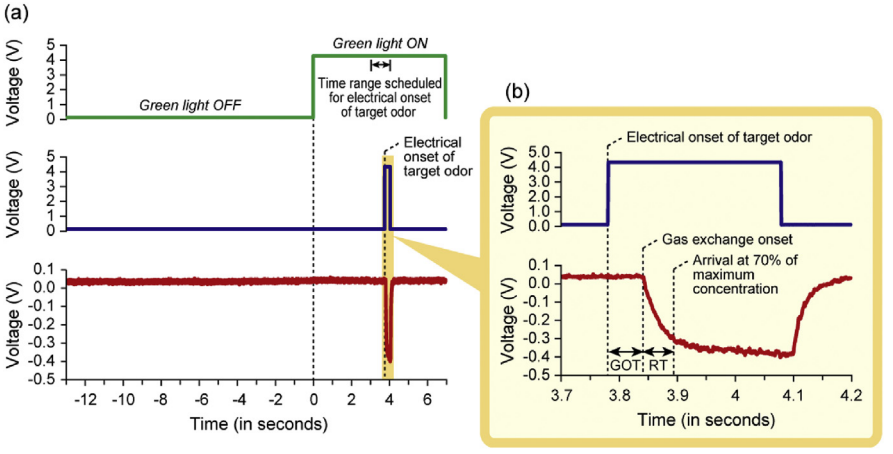

Fig. 4. Real-time monitoring of target odor under background odor and odorless air conditions. Changes in the voltage values on the basis of illuminating or extinguishing the green light (the upper row of [a]), output from the PC to the three-way solenoid valve (middle row of [a] and upper row of [b]), and output from the high-speed ultrasonic gas sensor (lower row of [a] and lower row of [b]) are shown. We defined gas onset time (GOT) as latency from electrical onset to gas exchange onset, and rise time (RT) as latency from gas exchange onset to arrival at 70% of maximum concentration (i.e., minimum voltage).

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3.2. Comparison of gas onset time and rise time of the target odor between the existing and expanded olfactometers

3.2.1. Procedure

The procedure for measurement using the expanded olfactometer was the same as that for measurement under the odorless air condition in Experiment 1. Measurement was conducted in 20 consecutive trials. In the measurement using the existing olfactometer, we used the gas wash bottle of the target odor unit in the expanded olfactometer. The rest of the procedure was the same as for the measurement using the expanded olfactometer.

3.2.2. Analysis

Gas onset time and rise time of the target odor in the existing and expanded olfactometers were calculated using the same procedure as in Experiment 1. To determine whether gas onset time and rise time of the target odor differed between both olfactometers, we conducted the unpaired *t*-test for each parameter.

3.2.3. Results

The gas onset times (means ±SD) of the target odor were 59.75 ± 0.91 ms for the existing olfactometer and 60.20 ± 1.11 ms for the expanded olfactometer. The rise times (means ±SD) of the target odor were 51.15 ± 3.95 ms for the existing olfactometer and 51.40 ± 2.96 ms for the expanded olfactometer. For gas onset time as well as rise time, there were no significant differences between the two olfactometers (unpaired *t*-test). These results demonstrate that performance related to the presentation of the target odor was equivalent between the existing and expanded olfactometers.

3.3. Experiment 3: Gas onset time and rise time of background odor

3.3.1. Procedure

Both odorless air and background odor (non-odorized air) were moistened when the gases pass through a humidifying module with deionized water. Consequently, it is impossible to discriminate between odorless air and background odor on the basis of the output from the high-speed ultrasonic gas sensor. Therefore, we removed the deionized water from the humidifying module, but poured 50 mL of deionized water into the gas wash bottle of the background unit, as in Experiment 1 and 2. These procedures produced a difference in the molecular weights of gases between odorless air (which did not contain water vapor) and background odor (which contained water

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vapor); thus, it was possible to discriminate between odorless air and background odor on the basis of the output from the high-speed ultrasonic gas sensor.

The flow rates of odorless air and background odor were both 7.2 L per minute, and the flow rate of exhaust was 5.8 L per minute. The U-tube manometer was used for adjustment of the flow rates, as in Experiment 1. Measurements of 20 trials for each gas were conducted by alternately repeating 10 s of odorless air presentation and 10 s of background scent presentation. Changes in voltage value based on output from the PC to the two-way solenoid valve of background odor unit, as well as the output from the high-speed ultrasonic gas sensor, were recorded at a sampling rate of 1,000 Hz. Electrical onset in this performance evaluation was defined as the time point at which the signal for switching from odorless air to background odor was output from the PC to the two-way solenoid valve of each unit.

In Experiment 1, because both odorless air and background odor (non-odorized air) were humidified, it was impossible to discriminate between these gases on the basis of the output from the high-speed ultrasonic gas sensor. Accordingly, the output from the high-speed ultrasonic sensor recorded during real-time monitoring of the target odor did not differ between the odorless air and background odor conditions (see Fig. 4). However, when non-humidified odorless air and humidified background odor (non-odorized air) were used, it was possible to discriminate between these gases on the basis of the output from the high-speed ultrasonic gas sensor. Therefore, in order to visualize a sequential exchange of gases switching from odorless air to background odor, target odor, background odor, and then to odorless air, changes in voltage value based on illuminating and extinguishing the green light, output from the PC to the three-way solenoid valve, and the output from the high-speed ultrasonic gas sensor were recorded at a sampling rate of 1,000 Hz.

3.3.2. Analysis

To calculate the gas onset time for each trial, we used 20,000 points of voltage value data (20 s $\times$ sampling rate 1,000 Hz) recorded over 20 s following electrical onset. Other procedures related to calculation of gas onset time and rise time were the same as in Experiment 1.

3.3.3. Results

An example of real-time monitoring of background odor with a high-speed ultrasonic gas sensor is shown in Fig. 5. The gas onset time and rise time (means $\pm$SD) of the background odor were 2041.00 $\pm$ 72.98 ms and 1668.75 $\pm$ 160.13 ms, respectively.

The gas onset time and rise time of background odor were on the order of seconds. On the other hand, the results of Experiment 1 and Experiment 2 demonstrated that

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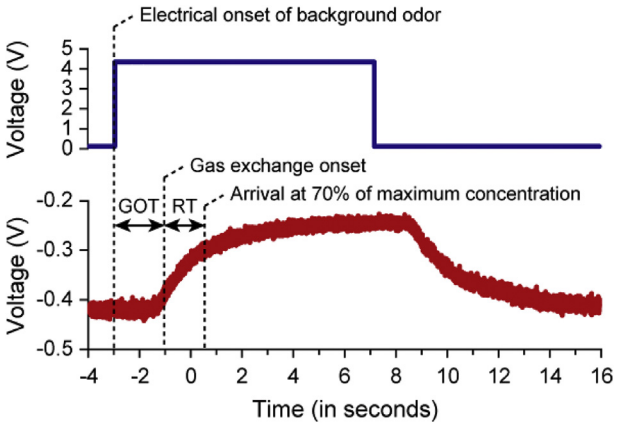

Fig. 5. Real-time monitoring of background odor. Changes of voltage values on the basis of output from the PC to the two-way solenoid valve of the background odor unit (upper row) and output from the high-speed ultrasonic gas sensor (lower row) are shown. GOT and RT represent gas onset time and rise time, respectively.

the gas onset time and rise time of target odor were on the order of milliseconds. We speculated that this large difference might depend on the distance between the parts involved in physically switching the gas flow paths and the high-speed ultrasonic gas sensor. Switching between odorless air and background odor was handled by the two-way solenoid valves of each unit. One gas flowed through the solenoid valve, whereas the other gas was stopped by the valve. The distance between the high-speed ultrasonic gas sensor and two-way solenoid valve was approximately 4.7 m. Switching between the background odor and target odor, or between odorless air and the target odor, was handled by a three-way solenoid valve. The three-way solenoid valve was responsible for determining which gas was presented in the nasal cavity of the participant and which gas was exhausted via the suction pump. However, the flow paths of the two gases were determined not by solenoid valves, but by a part shaped like a four-pronged fork close to the outlet of the olfactometer. The distance between the high-speed ultrasonic gas sensor and the fork-shaped part was approximately 3 cm. Based on the above, we expected that the gas onset time and rise time decreased when physical switching of gas flow paths was performed closer to the outlet of olfactometer. Incidentally, the expanded olfactometer required approximately 3.7 s (gas onset time + rise time) for the background odor, which was exchanged with the odorless air by switching the two-way solenoid valves of each unit, to reach equilibrium in the line of odorless air and background odor. The time required for odorless air, odorless nitrogen, or target odor to reach equilibrium in each line was also almost equivalent to the time required for the background odor.

An example of real-time monitoring of target odor under the background odor condition with a high-speed ultrasonic gas sensor is shown in Fig. 6. Because we used non-humidified odorless air and humidified background odor (non-odorized air), it

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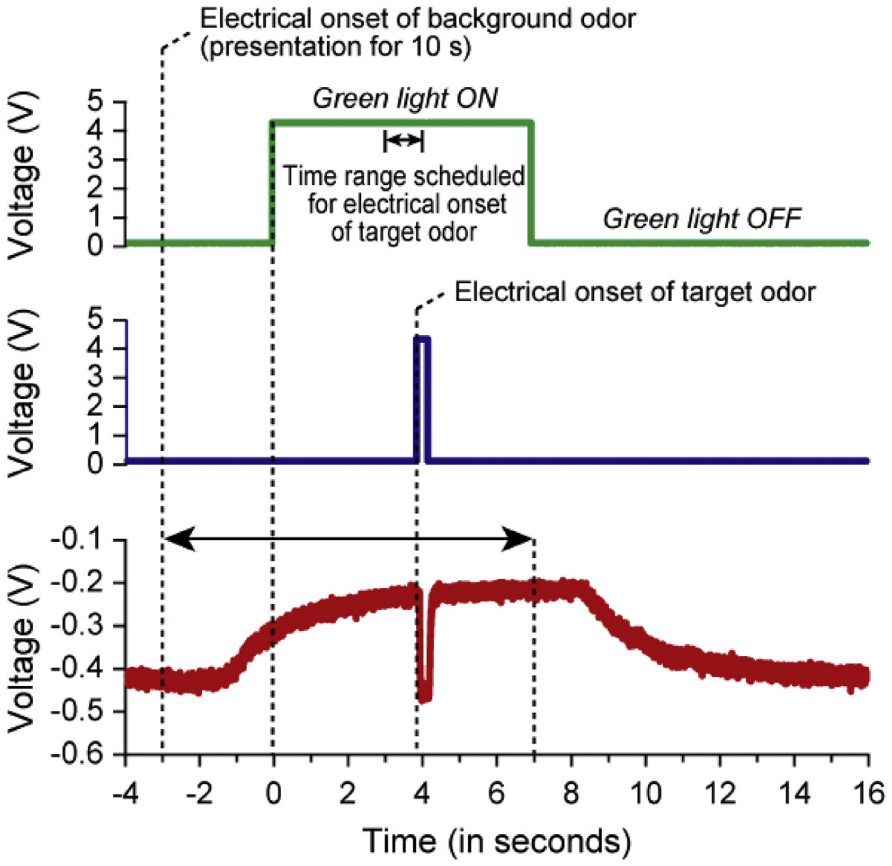

Fig. 6. Real-time monitoring of target odor under background odor condition. Changes in the voltage values on the basis of illuminating or extinguishing the green light (the upper row), output from the PC to the three-way solenoid valve (middle row), and output from the high-speed ultrasonic gas sensor (lower row) are shown.

was possible to visualize a sequential exchange of gases switching from odorless air to background odor, target odor, background odor, and then to odorless air. However, because the odorless air and background odor were humidified in the trial measurement of response time for detection of the target odor (described below), it was impossible to record the output from the high-speed ultrasonic gas sensor, as shown in Fig. 6.

4. Example

4.1. Participants

This study was conducted in accordance with the revised version of the Helsinki Declaration. All procedures in this study were approved by the ethical committee for ergonomic experiments of the National Institute of Advanced Industrial Science and Technology, Japan. We explained the experiments to each participant in advance

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of the study, and informed them of their right to cease participation even after their initial agreement to participate. Written informed consent was acquired from all participants. Four volunteers (one woman and three men) between the ages of 20 and 22 years old (mean age $\pm$SD $=$ 21.0 $\pm$ 1.2 years old) participated in the experiment.

4.2. Odors

Commercial black tea beverage (*Gogo-no-Kocha Oishii Muto* [Afternoon Tea Delicious Sugar-free]; Kirin Beverage, Tokyo) was used without dilution in order to generate the background odor. We poured 50 mL of black tea beverage into the gas wash bottle of the background odor unit. To confirm the perceived intensity of black tea odor, two experimenters smelled the outlet of olfactometer. In order to avoid changes in perceived intensity due to respiration, they smelled black tea odor presented for 7 s while holding their breath. Under these conditions, the perceived intensity of black tea odor was 1.5−2 on a six-point magnitude scale ('not detectable' [0], 'barely detectable' [1], 'weak' [2], 'moderate' [3], 'strong' [4], and 'very strong' [5]; see Saito, 1994).

To generate the target odor, we used lemon odorant (Lemon flavor 109; T&M, Chiba, Japan) diluted three-fold (v:v) with propylene glycol (special grade reagent; Wako Pure Chemical Industries, Osaka, Japan). We wrapped the tip of the Teflon tube inserted into the gas wash bottle of the target odor unit with a 2.5 cm $\times$ 2.5 cm absorbent cotton, which was fixed with a thin wire. We dropped 1 mL of diluted lemon odorant onto the absorbent cotton using a microsyringe. To confirm the perceived intensity of lemon odor, two experimenters smelled the outlet of the olfactometer. In order to avoid changes in perceived intensity due to respiration, they smelled lemon odor presented for 300 ms while holding their breath. Under these conditions, the perceived intensity of lemon odor was approximately 3 on a six-point magnitude scale (Saito, 1994).

4.3. Procedure

Measurement of reaction time was performed in a small room where external sound could be blocked. Although the door of the small room was closed during the measurement, experimenters could observe the inside of the room from the outside and interact with the participant via a camera and interphone. Additionally, to prevent the participant from predicting the presentation of the target odor depending on the sound of opening and closing of the solenoid valve, white noise was presented constantly throughout the measurement.

A green light was used as a warning for target odor presentation and fixation point. Specifically, a green LED light was emitted from a point approximately 150 cm from the face of the participant. In addition, in order to obtain responses related to

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detection of the target odor, a wooden cylinder (diameter 40 mm × height 84 mm) with a spring-type push button (diameter 85 mm) was used. The participant grasped the cylinder with the dominant hand, and was then asked to keep their thumb on the button during the measurement. When the button was pushed in by approximately 0.3 mm, a current flowed through the circuit due to conduction between the contacts, and a voltage of 5 V was generated at both ends of the resistor. The change in voltage value based on the pushing of the button was also processed at a sampling rate of 1,000 Hz, as were the changes in voltage value based on illuminating and extinguishing the green light, the output from the PC to the three-way solenoid valve, and the output from the high-speed ultrasonic gas sensor.

The flow rates of odorless air and background odor were 7.2 L per minute, the flow rates of odorless nitrogen and target odor were 5.0 L per minute, the flow rate of exhaust was 5.8 L per minute, the presentation time of the background odor was 10 s (actual time was 9.7 s; 0.3 s out of 10 s was spent to present the target odor), the presentation time of the target odor was 0.3 s (300 ms), and the presentation interval between target odors was approximately 20 s. The U-tube manometer was used for adjustment of the flow rates, as in Experiment 1. The green light was illuminated for 7 s per trial.

The timeline of the solenoid valve control in each trial under both the background odor and odorless air conditions was the same as the procedure in Experiment 1 (see Fig. 3). Background odor and odorless air conditions were presented in random order, and each participant underwent 40 trials (20 trials × 2 conditions). The participant was asked to stop breathing while the green light was turned on because the perceived intensity of the target odor might differ depending on whether it was presented in expiratory vs. inspiratory phases. Because the gases were presented at reasonable flow rates (7.2 L per minute for the background odor, and 5.0 L per minute for the target odor), participants could perceive the odor even if they stopped breathing. The target odor was presented while the green light was turned on, and the participant was asked to push the button as soon as they sensed a change in the olfactory environment.

4.4. Analysis

Using the same procedure for performance evaluation as in Experiment 1, we identified the gas exchange onset of the target odor. Next, the time for an odor to reach the participant's nasal mucosa from the high-speed ultrasonic gas sensor was calculated with reference to the distance between the center of the gas sensor and the tip of the Teflon tube attached to the gas sensor, the estimated distance between the tip of Teflon tube and the participant's nasal mucosa, the cross-sectional area of the Teflon tube, and the flow rate of the background odor. We added the time required for an odor to reach the participant's nasal mucosa from the high-speed ultrasonic gas sensor (i.e., 22 ms) to the gas onset of each trial, and thereby obtained the exact

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time at which the target odor arrived at the participant's nasal mucosa. Finally, we calculated the reaction time for detection of a target odor by subtracting the time at which it arrived at the participant's nasal mucosa from the time at which the participant began pressing the button.

To improve the accuracy of the analysis, data that deviated extremely from the average reaction time was excluded. More specifically, we calculated the average of reaction time and SD for each condition and each participant, and used only data corresponding to the average $-3 \times \mathrm{SD} \leq t \leq$ the average $+3 \times \mathrm{SD}$ (t represents reaction time) for analysis. These data was used to recalculate the average reaction time for each condition and each participant. Average reaction time was substituted for a trial in which data were missing (i.e., a trial in which there was no response because the participant did not push the button or that was regarded as an outlier). The number of non-response or outlier trials with for each participant under the background odor and odorless air conditions is shown in Table 1.

To determine whether reaction time for detection of the target odor differed between background odor and odorless air conditions, we performed two types of statistical analyses. First, we performed two-way repeated measure analysis of variance (ANOVA) for reaction time, with condition and trial number as within-subject factors. Simple effect tests were conducted on the basis of the significance of results obtained with ANOVA. Second, we performed Wilcoxon's signed-rank test using the average reaction time calculated for each condition and each participant. Because the number of participants was small ($n = 4$), a nonparametric test was adopted in the second analysis.

4.5. Results

The reaction times for each trial under the background odor and odorless air conditions are shown in Table 2. ANOVA revealed that neither the main effect nor the interaction was significant.

The average reaction time of each participant under the background odor and odorless air conditions is shown in Fig. 7. Wilcoxon's signed-rank test revealed a

Table 1. Number of trials with missing values for each participant under the background odor and odorless air conditions.

Participant	Background odor condition		Odorless air condition	
	Non-response	Outlier	Non-response	Outlier
A	1	0	4	0
B	0	1	1	1
C	2	0	0	1
D	0	0	0	0

Twenty trials were performed for each participant and each condition.

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Table 2. Reaction time for each trial under the background odor and odorless air conditions (means ±SD [ms]).

Trial	Background odor condition	Odorless air condition
1	717.78 ± 91.18	590.67 ± 113.49
2	678.25 ± 146.71	596.96 ± 158.65
3	682.75 ± 148.54	668.75 ± 78.99
4	664.00 ± 113.78	725.00 ± 132.24
5	792.00 ± 236.25	640.50 ± 80.67
6	616.25 ± 65.39	602.25 ± 136.97
7	742.75 ± 222.84	654.25 ± 62.02
8	720.00 ± 120.66	667.92 ± 127.87
9	723.00 ± 152.33	659.25 ± 101.97
10	621.50 ± 99.46	701.00 ± 134.32
11	734.75 ± 118.44	606.50 ± 62.51
12	834.75 ± 232.13	579.29 ± 109.12
13	733.75 ± 195.80	656.75 ± 109.65
14	640.50 ± 189.57	659.84 ± 68.84
15	677.78 ± 140.39	646.75 ± 67.84
16	671.50 ± 123.09	656.00 ± 38.50
17	654.42 ± 140.11	664.50 ± 195.85
18	750.58 ± 275.68	634.25 ± 55.16
19	581.25 ± 298.13	637.42 ± 127.95
20	555.25 ± 187.25	575.00 ± 136.92

marginally significant difference (defined as when $0.05 \leq p < 0.1$) between both conditions ($z = 1.83$, $p = 0.068$). This result indicated that the reaction time for detection of the target odor was marginally significantly longer under the background odor condition than under the odorless air condition. In other words, the background odor might affect detection speed of the target odor.

Because this study was a trial measurement of reaction time for detection of the target odor under background odor and odorless air conditions, the number of participants was small. If the number of participant increases could be increased, it is possible that the difference in reaction time between conditions could reach statistical significance.

5. Discussion

5.1. Current limitation and future issues

In daily life, it would be rare to encounter a situation in which a certain odor (target odor) was generated as a pulse while another odor (background odor) hangs in the

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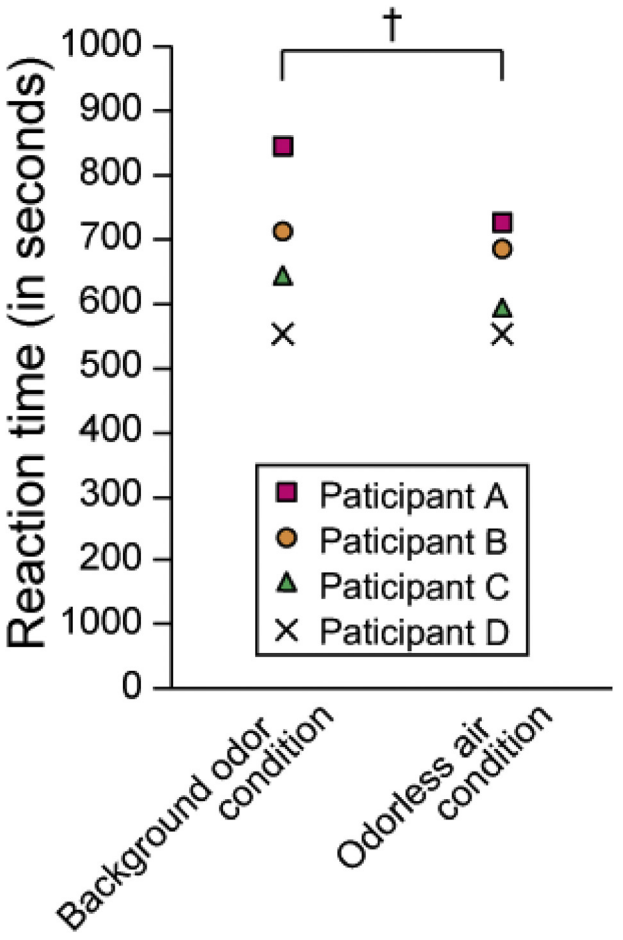

Fig. 7. Average reaction time of each participant under background odor and odorless air conditions. Average reaction time obtained from four participants under background odor and odorless air conditions are shown. Wilcoxon signed-rank test revealed that reaction time differed marginally significantly between both conditions ($z = 1.83$, $p = 0.068$). † $0.05 < p < 0.1$.

air. However, detection of target odors occurs not only in experimental situation but also in environmental context. Furthermore, the speed of detection of a target odor may depend on the olfactory environment such as the presence or absence of a background odor or the combination of background and target odors. When the olfactometer developed by Kobal and colleagues (Kobal, 1985; Kobal and Hummel, 1988) is used, target odor presentation can be temporally controlled with high accuracy (i.e., the rise time of approximately 50 ms). Accordingly, we conclude that when reaction time for detection of a target odor is measured in the presence or absence of a background odor using our expanded olfactometer, it will provide valuable insight into everyday perception of odor mixture.

In the expanded olfactometer, two units (odorless air and background odor units) were arranged in the middle of the odorless air line of the existing olfactometer. In other words, odorless air and background odor partially shared a flow path (see

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Fig. 1 (b)). Therefore, we cannot exclude the possibility that molecules of background odor were adsorbed inside the Teflon tube (i.e., flow path shared between both gases). However, in trial measurement of reaction time for detection of the target odor performed in this study, the presentation order between background odor and odorless air conditions were randomized. Under the background odor condition, to minimize the time that background odor passed through the flow path shared by background odor and odorless air, the two-way solenoid valve of background odor unit was opened only for 10 s. Under the odorless air condition, background odor did not pass through the flow path shared by both gases. Odorless air passing through the flow path shared by both gases contributed to flushing of the inside of the Teflon tube under the background odor and odorless air conditions. When the background odor condition was continuously presented for more than two trials, odorless air presentation for 10 s and background odor presentation for 10 s were repeated; i.e., the minimum flushing time of the line of odorless air and background odor in this study was 10 s. Before starting the trial measurement of reaction time, two experimenters smelled the gases presented at the outlet of the olfactometer and confirmed that background odor was not perceived when the two-way solenoid valve of background odor unit was closed. Additionally, after the end of measurement, none of participants reported that a background odor (black tea odor) was presented in all trials. However, depending on the type of background odor, it may be necessary to lengthen the time that odorless air passes through the flow path shared by background odor and odorless air, i.e., the flushing time.

5.2. Technical perspective

5.2.1. Extension of background odor and target odor units

The expanded olfactometer included one background odor unit and one target odor unit. In other words, one type of background odor and one type of target odor could be presented in each experimental session. By connecting multiple background odor units or multiple target odor units in parallel, it would be possible to present multiple odors (corresponding to the number of odor units) in each session.

In this study, we poured a liquid containing the odorant into the gas wash bottle of the background odor unit; alternatively, an absorbent cotton containing the odorant could be attached to the tip of the Teflon tube inserted into the gas wash bottle. For the target odor unit, as with the background odor unit, either an absorbent cotton or liquid containing the odorant could be used.

5.2.2. Humidifying module

Due to the structure of the expanded olfactometer, it is possible that molecules of a background odor could be mixed in deionized water to humidify the odorless air and

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background odor. We predict that increasing the number of background odor units would increase the probability that odorous molecules would contaminate deionized water. In order to address this issue, odorless air should be humidified before it reaches the upstream two-way solenoid valve of each unit, as shown in Fig. 8. More specifically, we propose that the humidifying module (see Fig. 1) common to the existing and expanded olfactometers is not used; instead, a humidifying module (i.e., gas wash bottle containing deionized water) could be placed between the mass flow controller and upstream two-way solenoid valve of each unit. When the position of the humidifying module is changed, both hydrophobic and hydrophilic odorants could be used because odorized air (background odor) never passes through the deionized water.

5.2.3. Simultaneous presentation of background odor and target odor

The expanded olfactometer had a mechanism for inserting the target odor into flow of the background odor. Strictly speaking, when this mechanism was employed, the

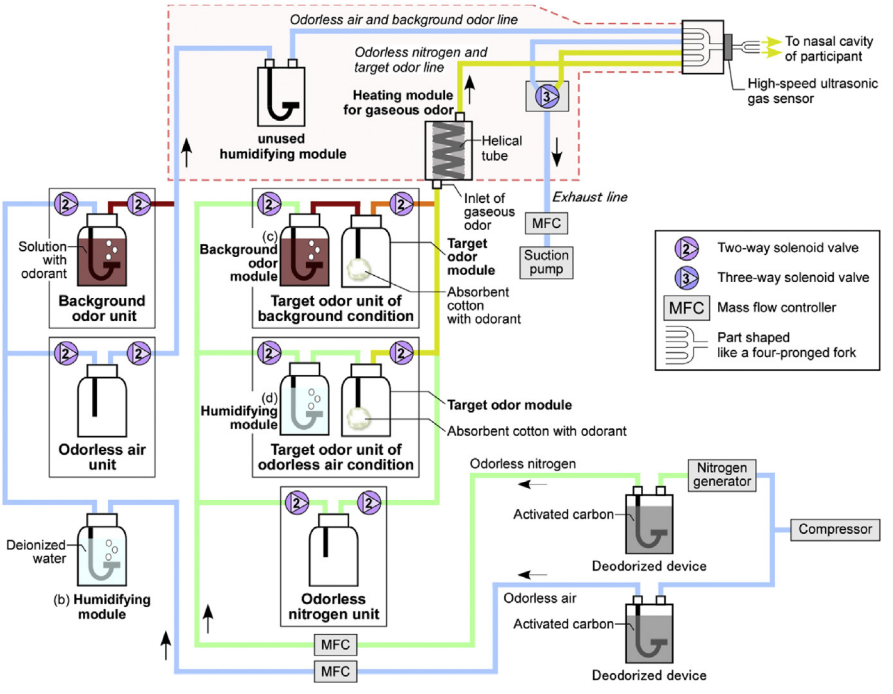

Fig. 8. Plan for improvement of expanded olfactometer. The humidifying module (a) common to existing and expanded olfactometer is not used. Instead, a humidifying module (b) is placed between the mass flow controller for the line of odorless air and background odor and the upstream two-way solenoid valves of each unit. Additionally, a background odor module (c) is placed between the upstream two-way solenoid valve of the target odor module for the background odor condition and its target odor module. When a solution with odorant is used to generate a background odor, a humidifying module (d) is placed between the upstream two-way solenoid valve of the target odor unit of the odorless air condition and its target odor module.

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background odor was replaced with the target odor for a very short time (several hundred milliseconds). Because we switched from the background odor to target odor, and then back to the background odor, the background odor was not presented while the target odor was presented to the participant's nasal cavity. In other words, the mechanism of the expanded olfactometer cannot achieve simultaneous presentation of background and target odors. Therefore, we propose a future improvement of target odor unit, as shown in Fig. 8. By placing a background odor module between the upstream two-way solenoid valve of target odor unit and its target odor module, a mixture of the background and target odor can be generated. When a solution with odorant is used to generate a background odor, a humidification module should be placed between the upstream two-way solenoid valve of the target odor unit of the odorless air condition and its target odor module, in order to achieve the same degree of humidity in the target odor under the odorless air condition as in the target odor under the background odor condition.

6. Conclusion

In this study, we developed an olfactometer for measuring reaction time to target odors during the presentation of background odors. Accuracy related to the presentation of the target odor was equivalent between the background odor and odorless air conditions, as well as between the existing and expanded olfactometers. In other words, even when an attachment was added to the existing olfactometer, performance related to the presentation of the target odor did not deteriorate. Four volunteers participated in a trial measurement of reaction time for detection of the target odor under the background odor and odorless air conditions using the expanded olfactometer. Reaction time was marginally significantly longer under the background odor condition than under the odorless air condition. In order to more rigorously examine effect of a background odor on detection speed of the target odor, we intend to increase the number of participants in the near future.

To insert a target odor into a flow of background odor as a pulse, it is important not only to carefully design the structure of the olfactometer, but also to control the solenoid valves with appropriate timing. Given the gas onset time and rise time of the background odor, it is necessary to determine the electrical onset of the background odor.

Declarations

Author contribution statement

Naomi Gotow, Tatsu Kobayakawa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Ayaka Hoshi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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