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Comparison of the Orthonasal and Retronasal Detection Thresholds for Carbon Dioxide in Humans

Johannes Melzner[1], Thomas Bitter[1], Orlando Guntinas-Lichius[1], Reiner Gottschall[2], Mario Walther[3] and Hilmar Gudziol[1]

[1]Department of Otorhinolaryngology, University Hospital Jena, Lessingstraße 2, 07743 Jena, Germany, [2]Clinic for Anesthesiology and Intensive Care Medicine, University Hospital Jena, Erlanger Allee 101, Jena, Germany and [3]Institute of Medical Statistics, Computer Sciences and Documentation, Friedrich-Schiller-University Jena, Bachstraße 18, 07743 Jena, Germany

Correspondence to be sent to: Hilmar Gudziol, Department of Otorhinolaryngology, University Hospital Jena, Lessingstraße 2, 07743 Jena, Germany. e-mail: hilmar.gudziol@med.uni-jena.de

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Abstract

Several studies have investigated the orthonasal detection threshold for carbon dioxide (CO_2) in humans. The aim of current study was to investigate whether 24 healthy young subjects exhibited differences of CO_2 detection thresholds during orthonasal or retronasal stimulation. As nasal mucosa is believed to desensitize to CO_2 concentrations at or below 4% (v/v) during expiration, the second aim of the study was to explore the influence during nasal versus oral breathing on the detection thresholds. CO_2 stimuli of varying concentrations and a duration of 1000 ms were applied with an air-dilution olfactometer in either the anterior nasal cavity or the nasopharynx during nasal respectively oral breathing. In these 4 conditions, the mean CO_2 detection thresholds using the staircase forced-choice procedure were between 3.9% and 5.3% (v/v). Statistical analysis revealed a significant difference between orthonasal and retronasal stimulation. The CO_2 detection threshold was lower in retronasal stimulation. The nasopharyngeal mucosa is more sensitive to perithreshold CO_2 stimuli than the nasal mucosa. The breathing route had no influence on the detection thresholds. The results of this study indicate that the natural contact of the nasal mucosa with approximately 4% (v/v) CO_2 during nasal expiration does not influence CO_2 detection thresholds.

Key words: carbon dioxide (CO_2), detection threshold, orthonasal, retronasal, smelling

Introduction

Many people regard the nose as having only a single sensory role: the detection of odors. In animals, the olfactory system not only identifies food and assays its quality but also detects information about reproductive status, gender, and genetic identity, thus making an important contribution to controlling social interaction and regulating reproductive behavior (Fleischer et al. 2009). However, nasal sensations depend not only on the sense of smell but also on the activity of branches of the trigeminal nerve. Few chemosensory stimulants produce exclusively olfactory or trigeminal sensations (i.e., itchy, burning, or stinging sensations); indeed, the vast majority of chemosensory stimulants possess characteristics of both odor and irritation (Doty et al. 1978). Inhalation of irritating chemical stimuli activates trigeminal nerve endings and triggers protective reflexes, such as apnea and sneezing.

Carbon dioxide (CO_2) is a selective trigeminal stimulant with very low olfactory potency (Kobal 1985; Thuerauf et al. 1991). As trigeminal nerve endings are spread over the nasal mucosa, CO_2, in contrast to odors without any trigeminal sensation, is not only detected in the olfactory cleft but also in the entire nasal cavity and the nasopharynx. The molecular model of CO_2 detection is based on the enzyme carboanhydrase (CA). It is assumed that CO_2 hydrates and then releases a proton in a reaction catalyzed by CA (Komai and Bryant 1993; Hummel et al. 2003; Shusterman and Avila 2003). The intracellular accumulation of protons activates chemosensory nociceptive afferents by increasing the cation membrane conductance (Lingueglia et al. 1997; Waldmann et al. 1997). An alternative mechanism for CO_2 detection below expiratory levels was identified in bullfrogs whose olfactory receptor neurons are activated by CO_2

(Coates 2001). There are indications in the literature that orthonasal CO_2 stimulation activates a subset of olfactory neurons in some mammals, thereby regulating breathing inhibition or innate animal behavior (e.g., seeking food or avoiding a stressful environment) (Youngentob et al. 1991; Hu et al. 2007).

Several studies have reported a difference between orthonasal and retronasal perception of odors (Heilmann and Hummel 2004; Bender et al. 2009). However, little is known about the orthonasal detection threshold for CO_2, and nothing is known about the retronasal CO_2 detection threshold in humans. Reported thresholds for orthonasal CO_2 detection range from approximately 10% to 20% (v/v) in healthy young subjects, depending on the duration of the stimulus and the overall procedure (Stevens and Cain 1985; Thuerauf et al. 2002; Wise et al. 2004; Frasnelli and Hummel 2005; Frasnelli et al. 2006; Andersson et al. 2009; Frasnelli et al. 2010). Therefore, this study aimed to investigate the perception of CO_2 through the orthonasal and retronasal routes. Furthermore, oral breathing is presumed to result in lower intranasal thresholds for CO_2. As the nasal mucosa has no contact with expiratory end-tidal CO_2 concentrations during oral breathing, we predicted that the CO_2 sensing receptors do not desensitize.

When using olfactory stimuli (i.e., chocolate and lavender), the orthonasal detection threshold is lower than the retronasal detection threshold (Heilmann and Hummel 2004). Therefore, another objective of the current study was to investigate the differences between orthonasal and retronasal CO_2 detection thresholds. A technique reported by Heilmann and Hummel (2004) was used for stimulation, which allows the retronasal application of odors or irritants in a precisely defined manner without concomitant gustatory stimulation. This technique is based on the release of CO_2 directly into the nasopharynx during complete velopharyngeal closure. When employing this technique, the released CO_2 is not allowed to reach the oral cavity as a taste sensation could otherwise be evoked and result in confusion with nasal trigeminal irritation (Chandrashekar et al. 2009).

Materials and methods

This study was conducted according to the Declaration of Helsinki on biomedical research involving human subjects (Somerset West amendment; World Medical Association, 1996) and was approved by the ethics committee of the University of Jena Medical School. Twenty-five subjects were included between June and August 2009. Written informed consent was obtained from all subjects prior to their inclusion in the study. Participants were healthy, nonsmoking normosmic students with a mean age of 24.2 years (range: 18–30 years, including 16 women and 8 men). Only subjects not suffering from any medical disorder were included. Subjects underwent a detailed physical examination by an ear, nose, and throat (ENT) specialist to exclude nasal obstruction and nasal pathology. Normosmia was ascertained by means of the validated Sniffin' Sticks test kit, which included orthonasal tests for *n*-butanol detection threshold, odor discrimination, and odor identification (Hummel et al. 1997, 2007). The mean TDI (threshold, discrimination, and identification) score was 36.8 (range: 33–43.2). The physical examination by the ENT specialist was conducted in a separate session prior to the actual experiment. For measurements of orthonasal and retronasal detection thresholds, subjects were comfortably seated in an air-conditioned room.

Stimulation device

Stimuli were presented either orthonasally or retronasally by means of a computer-controlled air-dilution olfactometer (OM2s; Burghart). This apparatus allowed the application of repeated stimuli of a stable concentration. Concomitant somatosensory or other sensory stimuli were avoided by embedding the stimuli into a constant flow of odorless humidified air at a controlled temperature (36 °C, 80% relative humidity, total flow 8 L/min) (Kobal 1981). A tube connected to a differential pressure manometer (Sensing and Control, Honeywell Inc.) was placed into the vestibulum of the left nostril to record nasal breathing or to check velopharyngeal closure. Therefore we can guarantee the application of stimuli without dilution through expiratory air from the oropharynx. The rest of the left vestibulum was sealed with plastic, which effectively prevented nasal respiration through the left nostril.

For orthonasal stimulation, a tube from a nose adapter size 2 (Atmos Medizin Technik GmbH & Co. KG; 4.0 mm outer diameter, 2.0 mm inner diameter) was placed approximately 1.0 cm into the right vestibulum of the nose. For retronasal stimulation, CO_2 was released into the nasopharynx cranial to the soft palate (ca. 8.5 cm from the naris) through tubing via the lower nasal meatus (Figure 1). Tubes were cut from a sterile suction catheter (Ch. 08; Tyco Healthcare/Kendall). The tubes were attached to the upper lip using adhesive tape and connected to the exit of the olfactometer (Figure 2). To avoid mechanical irritation of the nasal mucosa during orthonasal stimulation, the tube for retronasal stimulation was only placed after the tube for orthonasal stimulation

Figure 1 Position of tubes during orthonasal (left) and retronasal (right) stimulation; airstream is shown as a dashed line.

was removed from the nose. The interstimulus interval (ISI) was 40 s, and stimuli of 1000 ms duration were applied. The patients stopped breathing during all the stimulus presentations.

The experiment was designed to include 4 test conditions, all of which were conducted on the same day and in the same sequence as follows:

1. orthonasal stimulation and nasal breathing (OSNB) during the ISI (no velopharyngeal closure during ISI and stimulation);
2. orthonasal stimulation and oral breathing (OSOB) during the ISI (velopharyngeal closure during ISI, no closure during stimulation);
3. retronasal stimulation and nasal breathing (RSNB) during the ISI (velopharyngeal closure during stimulation, no closure during ISI); and
4. retronasal stimulation and oral breathing (RSOB) during the ISI (velopharyngeal closure during stimulation and the ISI).

The participants were trained to perform a special breathing technique called velopharyngeal closure (Kobal 1981). By lifting the soft palate, respiratory airflow in the nasal cavity was avoided through physical separation of the nasal cavity from the oral cavity. The complete closure and opening of the soft palate were checked continuously by means of a differential pressure manometer connected to a tube in the left nostril and corrected if necessary. Velopharyngeal closure was performed while subjects were breathing through the oral route over the period of the ISI to avoid respiratory airflow over the nasal mucosa. Moreover, velopharyngeal closure was accomplished during retronasal stimulation, thereby guaranteeing airflow from the posterior to the anterior part of the nose.

Stimuli were presented in triplets consisting of 2 blanks and one stimulus with CO_2. Following each triplet, subjects had to indicate which of the 3 stimuli enclosed CO_2. Stimuli were presented in ascending order, starting with 1.0% (v/v) CO_2. The maximal obtainable concentration was 35.1% (v/v) CO_2. Concentrations were increased in steps of approximately 2% (v/v) CO_2. Thresholds were measured by means of a staircase forced-choice procedure, starting at the lowest concentration. If the subject did not perceive the irritant, a higher concentration was used. After the subject had perceived a certain concentration step twice (turning point 1), the CO_2 concentration was lowered again until the irritant was no longer detected (turning point 2). CO_2 concentrations were then increased until the irritant was detected 2 more times (turning point 3). Altogether, 7 turning points were measured. The average of the last 4 concentration steps (turning points 4, 5, 6, and 7) was used as a threshold estimate. Data acquisition lasted for approximately 2 h. To ensure accuracy, CO_2 concentrations were checked by an anesthetic apparatus (Datex-Engstrom AS/3 ADU; GE Healthcare) at the outlet of the olfactometer similar to the situation in vivo (Table 1). Measurements showed good agreement between calculated and measured concentrations. All subjects described their sensations at turning points 5 and 7 in each test condition. Therefore, subjects were given 4 descriptors (“itchy,” “burning,” “stinging,” and “sparkling”) beforehand. After each stimulus triplet, they were asked which of those descriptors fitted best for the perceived sensation.

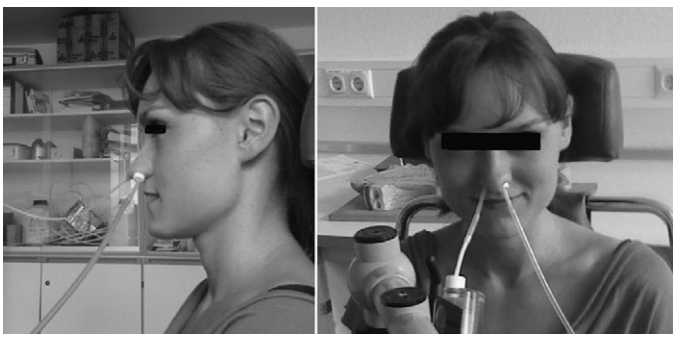

Figure 2 Setup of measurements with the tube for stimulation in the right nostril and the tube to record nasal breathing in the left nostril. Left picture: mouth open and oral breathing. Right picture: mouth closed and nasal breathing. The tube for retronasal stimulation was only placed in the nasopharynx after orthonasal stimulation.

Statistical analyses

The software package IBM SPSS statistics 19.0 (IBM corporation) was used for statistical analyses. Linear mixed model with compound symmetry with 2 independent within-subject factors, one being “presentation route” (1: orthonasal, 2: retronasal) and the other one “breathing” (1: oral breathing, 2: nasal breathing) was chosen for statistical analysis. To determine whether the breathing routes or the stimulation routes have any effects on detection thresholds, fixed effects were analyzed. Exploratory data analysis together with tests of normality revealed normality of the 4 test conditions if the measurement with a CO_2 detection threshold of 13.3% (v/v) (Table 2, subject 3) during OSNB was excluded. Missing

Table 1 Calculated and measured CO_2 concentrations among subjects

Calculated concentrations(v/v%)	Mean measured concentrations (v/v%)
1.0	1.1 ± 0.1
3.0	3.0 ± 0.1
5.0	5.2 ± 0.1
7.0	7.2 ± 0.0
9.1	9.3 ± 0.0
11.0	11.3 ± 0.1
13.0	14.3 ± 0.1
15.0	15.0 ± 0.0

values concerned additionally the 2 floor measurements and those if subjects had some troubles with the procedure (Table 2). So the statistics were done with different numbers of measurements (OSNB: $n = 22$, OSOB: $n = 23$, RSNB: $n = 22$, RSOB: $n = 20$). The alpha level was set at 0.05 (2-tailed).

Results

All detection thresholds in the 4 test conditions are shown in Table 2. The highest mean of detection thresholds, 5.3% (v/v) CO_2, was found during OSNB. The lowest was detected during RSOB, 3.9% (v/v) CO_2 (Table 2). The presentation route exhibited a significant effect on the detection thresholds ($F = 8.251$, $P = 0.006$). Thresholds during retronasal stimulation were lower than during orthonasal stimulation. The estimated means of CO_2 detection thresholds during retronasal stimulation and orthonasal stimulation were 4.1%, respectively 4.9% (v/v) CO_2. The breathing routes and the interaction of stimulation and breathing routes were without any effects ($F = 3.668$, $P = 0.060$; respectively, $F = 0.423$, $P = 0.518$).

Sensations at turning points 5 and 7 were recorded as follows: itchy (57 times), stinging (18 times), sparkling (16 times),

Table 2 CO_2 threshold values (v/v %) for all subjects under all test conditions

Subject	OSNB	OSOB	RSNB	RSOB
1	2.5	2.0	6.0	3.5
2	5.0	3.0	3.0	[a]Floor effect
3	[a]13.3	4.5	3.5	4.0
4	[a]Floor effect	2.0	4.0	3.0
5	2.0	2.5	2.5	4.0
6	6.5	6.5	5.0	4.0
7	5.0	2.0	[a]Heavy sneezing attack	[a]Heavy sneezing attack
8	7.0	5.5	2.5	4.0
9	5.0	5.0	3.5	6.0
10	7.0	6.0	6.0	3.5
11	4.0	4.5	4.0	4.0
12	6.0	5.0	3.5	2.5
13	3.0	4.0	2.5	2.0
14	6.5	4.5	4.0	4.5
15	5.0	4.0	3.0	3.5
16	3.5	6.0	6.5	4.0
17	4.0	4.0	3.5	2.5
18	6.0	3.5	4.5	4.0
19	4.0	7.0	5.5	5.5
20	3.0	3.0	5.5	6.0
21	8.4	7.6	5.0	4.5
22	7.0	[a]Veloph. closure impossible	5.0	[a]Veloph. closure impossible
23	8.1	6.0	[a]Heavy sneezing attack	[a]Heavy sneezing attack
24	6.5	4.5	3.5	2.0
Mean threshold	**5.3**	**4.5**	**4.2**	**3.9**
Standard deviation	**1.8**	**1.6**	**1.3**	**1.2**
Minimum	**2.0**	**2.0**	**2.5**	**2.0**
Maximum	**8.4**	**7.6**	**6.5**	**6.0**

[a]Excluded from statistical analysis.

and burning (4 times). Stinging sensations were recognized at 3.0%, 5.0%, 7.0%, 9.1%, and 11.0% (v/v) CO_2 (Table 3).

Discussion

The mean CO_2 detection thresholds (Figure 3) measured during OSNB respective OSOB in the current study is quite low (5.3 ± 1.8% [v/v]; 4.5 ± 1.6% [v/v]) when compared with the mean thresholds of previous studies (Thuerauf et al. 2002: 20.6 ± 9.6% [v/v]; Andersson et al. 2009: 20.6 ± 8.5% [v/v]; Frasnelli et al. 2010: 12.5 ± 0.5% [v/v]). This variation might be caused by methodological differences. Frasnelli et al. (2010) assessed the detection thresholds for CO_2 in 48 young healthy subjects using the single staircase method without any blanks; the lowest concentration used for stimulation was 10% (v/v). The authors critically discussed that a perithreshold result rather than a true threshold result was obtained; the detection threshold should be lower due to observed floor effects using 10% (v/v) CO_2 as the lowest stimulus concentration (Frasnelli et al. 2010). Another limitation of the aforementioned study is the fact that the authors forgot to disclose the stimulus duration. An increase in stimulus duration has been shown to lead to larger intensity ratings with the same suprathreshold stimulus concentration (Cometto-Muniz and Cain 1984). This was also found at threshold levels when the stimulus duration was varied over a wide range of fixed concentrations (Wise et al. 2004).

In a comparable study, Thuerauf et al. (2002) reported a detection threshold of 20.6 ± 9.6% (v/v) CO_2. The detection thresholds of 10 young healthy subjects were determined with ascending stimulus intensity relative to blanks (ascending method of limits, AMLs). Test series initiated at 4% (v/v) CO_2 and were increased by steps of 2% (v/v) CO_2. The stimulus duration was 1000 ms and the ISI was 40 s. The AML procedure has been shown to result in higher thresholds relative to the staircase method (Linschoten et al. 2001). Moreover, Doty et al. (1995) showed that obtained thresholds using the AML procedure are less reliable than those using the staircase procedure. This could be another reason for the discrepancy to the results of our study.

In another study, Andersson et al. (2009) reported a detection threshold of 20.6 ± 8.5% (v/v) CO_2 also using an AML procedure; the stimuli lasted only 200 ms. In that study, Andersson et al. (2009) identified a CO_2 detection threshold that was nearly 4 times higher than the mean threshold in our study. However, the stimulus duration was only one-fifth the stimulus duration used in the present study (1000 ms vs. 200 ms; detection thresholds: 5.3% vs. 20.6% [v/v] CO_2, respectively). Our results indicate that the quasi-linear relationship between stimulus duration and suprathreshold CO_2 concentrations relative to subject ratings (Frasnelli et al. 2003) also exists at threshold levels with fixed duration time and varying CO_2 concentrations.

Wise et al. (2004) asserted that a 2-fold increase in duration time results in less than a 2-fold decrease in concentration at threshold levels. Additionally, Wise et al. (2004) ascertained that stimulus durations below 2000 ms did not allow subjects to detect CO_2 concentrations lower than 10% (v/v). However, our results do not confirm this finding. In all of our test conditions, with the exception of one outlier in the first test condition, the detection threshold values were below 10% (v/v) CO_2 with a stimulus duration time of 1000 ms (Table 2).

The current study revealed significant higher values for orthonasal stimulation than for retronasal stimulation. The breathing routes did not influence this finding. Our search of the literature did not uncover values for retronasal CO_2 detection thresholds. Heilmann and Hummel (2004) detected a significant difference between orthonasal and retronasal stimulation when using olfactory stimuli (i.e., chocolate and lavender) in the absence of trigeminal stimulation. Thresholds for orthonasal stimulation were significantly lower than those for retronasal stimulation. Large differences in odor perception produced by subtle changes

Table 3 Sensations of subjects at turning points 5 and 7, including one subject with descriptor (1) and (4) during OSNB, 2 subjects with descriptor (1) and (3) during OSOB, one subject with descriptor (1) and (4) during OSOB and 2 subjects with descriptor (1) and (4) during RSOB

Sensations	Number of subjects			
	Orthonasal stimulation		Retronasal stimulation	
	Nasal breathing	Oral breathing	Nasal breathing	Oral breathing
Itchy (1)	12	16	14	15
Burning (2)	1	1	1	1
Stinging (3)	7	5	4	2
Sparkling (4)	5	4	3	4

The other subjects consistently used one descriptor during each test condition.

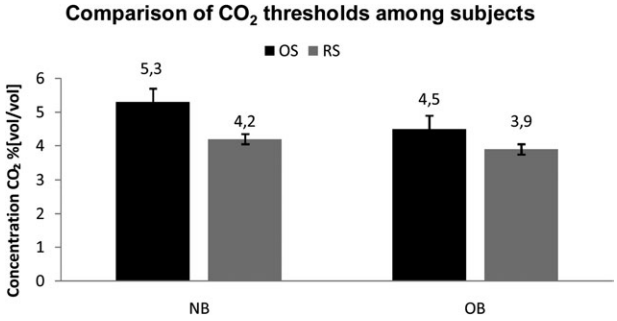

Figure 3 Comparison of CO_2 mean thresholds among subjects. Gray bars: orthonasal stimulation, black bars: retronasal stimulation; left side: nasal breathing, right side: oral breathing.

in the airstream in the region of the olfactory cleft were believed to be one reason for these significant differences (Damm et al. 2003).

Several studies support the idea of functional different areas within the nasal mucosa with regard to trigeminal sensitivity (Frasnelli et al. 2004; Scheibe et al. 2006, 2008). By recording negative mucosal potentials or chemosomatosensory event-related potentials, these studies underline the idea that the anterior part of the nasal mucosa is more sensitive to suprathreshold stimuli than the posterior part. In opposite to our investigation, all these studies used unexceptionally suprathreshold stimuli (30, 40 or 60% [v/v] CO_2). We suppose that the anterior part of the nasal mucosa is more sensitive to noxious suprathreshold stimuli, whereas the retronasal mucosa is more sensitive to perithreshold stimuli. We speculate that the trigeminal nerve endings within the mucosa of the inner nose play a sentinel role against strong irritants in the inspiration air. The second line of defense against weak perithreshold irritants in the inhaled air is situated in the nasopharynx, where the sensory sensitivity for these weak stimuli is higher than in the nasal cavity, possibly because the sensory part of the glossopharyngeal nerve also supplies the posterior wall of the nasopharynx. In the oropharyngeal innervation area of the ninth cranial nerve ingested weak capsaicin solutions resulted in stronger irritation than in the oral mucosa innervated by the trigeminal nerve (Rentmeister-Bryant and Green 1997). Thus, the higher sensitivity of the pharyngeal mucosa also might play a role during ingestion of spicy food and carbonated beverages.

Statistical analysis revealed no significant difference between the threshold values dependent of breathing route. Driving expiratory air over the nasal and nasopharyngeal mucosa with end-tidal CO_2 concentrations also had no influence on the CO_2 detection threshold. As thresholds during OSNB are as low as thresholds during OSOB, we suppose that the 2 s during inspiration are sufficient for a possible resensitization.

All the participants in this study experienced perithreshold sensations, such as itching, sparkling, burning, or stinging (Table 3). The stinging sensation is believed to be processed mainly by Aδ-fibers, whereas the burning sensation appears to be processed mainly by C-fibers (Finger et al. 1990). At low CO_2 concentrations, stimuli appear to activate mainly Aδ-fibers (Hummel et al. 1994). CO_2 appears to activate chemosensory nociceptive afferents via the intracellular accumulation of protons (Komai and Bryant 1993), which in turn leads to an increase in cation membrane conductance. A vanilloid receptor 1 (TPVR1) activated by protons was identified in sensory afferents of trigeminal and dorsal root ganglia, too (Caterina et al. 1997). Another means of activating nociceptors includes the acid-sensing ion channels (Lingueglia et al. 1997). We speculate that different trigeminal receptors may be engaged in discriminating different CO_2 concentrations.

Conclusions

Taken together, the results of the present study do not support the hypothesis that different CO_2 detection thresholds are associated with oral versus nasal breathing. In opposite to studies using olfactory stimuli, the CO_2 detection thresholds during retronasal stimulation were lower than thresholds during orthonasal stimulation. As thresholds during OSNB are as low as thresholds during OSOB, we suppose that the end-tidal CO_2 concentration has no desensitizing effect.

The CO_2 detection threshold reported herein is low but is in line with threshold values reported in the literature. The stimulus duration used in this study is 5 times higher than the stimulus duration used by Andersson et al. (2009), but the CO_2 detection threshold is more than one-fifth of the threshold measured by Andersson. Therefore, we conclude that the quasi-linear relationship between stimulus concentration and duration for suprathreshold stimuli identified by Frasnelli et al. (2003) also applies to perithreshold stimuli as found here and by Wise et al. (2004). Nevertheless, in contrast to the findings of Wise et al. (2004), the results of the current study show that CO_2 detection thresholds below 10% (v/v) are possible when using a stimulus duration of 1000 ms.

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