Retronasal but not oral-cavity identifications of air-phase trigeminal stimuli

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ABSTRACT:

Single concentrations of six odorants (eugenol, heptyl alcohol, nonanal, 1-octanol, dl-menthol, valeric acid) were selected to be trigeminal stimuli, based upon previous studies done through anosmics (individuals that lack a functional olfactory system). The stimuli were presented in random order three time each in vapor-phase either retronasally or oral-cavity-only. They were identified on a digital computer by 20 subjects (ages 18 to 35, 9 females). Retronasal presentations were produced by inhaling via the mouth with a nose clip closing the nostrils, and then removing the nose clip and exhaling from the nose. Oral-cavity-only presentations were produced by inhalation via the mouth with a nose clip closing the nostrils, and exhalation from the mouth with the nose clip remaining in place. This study investigates if subjects could identify odorants when restricted to the oral-cavity-only using the same identifiers of the odorants when presented retronasally. RESULTS: Median percent retronasal correct identifications [correct identification terms are shown in brackets] were: eugenol, 100% [cloves or spice]; heptyl alcohol, 67% [cleaner]; nonanal, 58% [citrus or floral]; 1-octanol, 71% [citrus or cleaner]; dl-menthol, 100% [ointment or peppermint]; valeric acid, 67% [rancid or sweat]. Median percent correct oral-cavity-only identifications were all 0%, except for dl-menthol, for which percent correct oral-cavity-only median correct identification was 67%. A Friedman Non-Parametric ANOVA statistical analysis showed significant difference between odorants presented oral-cavity-only versus retronasal. Pairwise comparisons showed significant differences between odorants presented oral-cavity-only versus retronasal for all odorants except for dl-menthol. CONCLUSIONS: Many vapor-phase 'trigeminal' odorants can be identified only when access to the nasal cavity (retronasal) occurs, but substantial correct identification of vapor-phase dl-menthol also occurs when restricted to the oral cavity (oral-cavity-only). Odorants similar to dl-menthol may contribute to flavor from both the oral and nasal cavities.

INTRODUCTION:

There are two primary ways that odorants (volatile chemical compounds that are carried in the air-phase and can be smelled at some concentration) can reach the olfactory mucosa. One of the pathways is through a retronasal route. The retronasal route involves odorants moving during an exhalation from the mouth via the oropharynx and nasopharynx, through the posterior nares (chonae), into the nasal cavities (Halpern, in press 2007). The odorants are thus allowed to flow through the nasal cavities during an exhalation, exiting through the anterior nares. A small portion of the odorants reaches the olfactory epithelium which is very dorsal and relatively posterior in the nasal cavities (Zhao, et al., 2004). However, the exact percentage of odorants from a retronasal route that reach the epithelium is yet unknown. The second way that odorants can reach the olfactory mucosa is called orthonasal olfaction, which involves odorants entering via the anterior nares (i.e., nostrils) to flow through the nasal cavities, with only a small portion of the odorants, about 10%, reaching the olfactory epithelium (Rawson, N. E., 2000). It is known that it takes a higher concentration of odorants for detection or identification of odorants when presented retronasally than orthonasally and that this difference is not fully explained by gross fluid dynamic and flow rate differences (Heilmann and Hummel, 2004, Halpern, in press 2007) Rather it is due to differences within the nasal cavity (Zhao et al., 2004). When asked to identify an odorant, previous studies report that orthonasal and retronasal identification were often comparable (Pierce and Halpern, 1996; Sun and Halpern, 2005).

The area represented by the human olfactory epithelium is quite small (2.5cm^2 wide) and contains approximately 40 million olfactory receptor cells. Along with

olfactory receptor cells, the epithelium also consists of basal cells and supporting cells. Basal cells are stem cells that give rise to new olfactory receptor cells. Support cells**,** also called sustentacular cells, which have numerous microvilli and secretory granules, empty their contents onto the mucosal surface and are found scattered among the receptor cells. These cells along with other secretory cells (Bowman's glands) help produce the mucus that lines the nasal cavity (Vokshoor and McGregor, 2006).

Olfactory receptor neurons (ORN) have dendrites eventually forming an olfactory knob from which 5-20 cilia protrude outward into mucus, where odorants interact with the cilia and provide the transduction surface for odorous stimuli. The basal ends of ORN have small-diameter, unmyelinated axons which project to the central nervous system (the olfactory bulb) (Purves et al., 2004). Recent evidence in mice has shown a random distribution on the epithelium of ORN that respond to various odorants. However, all mouse ORNs expressing the same receptor type project to the same glomerulus or multiple glomeruli in the olfactory bulb (Bozza et al., 2002).

The trigeminal system is a separate sensory system from the olfactory system. The trigeminal nerve (cranial nerve V) innervates the nasal cavity, including the olfactory epithelium and the oral cavity (Halpern, 2007), as well as other head-related regions (such as the face, scalp and cornea). These nerve endings are suspected to be responsible for tactile, pressure, pain, and temperature detection in the areas of the mouth, eyes and nasal cavity (Leffingwell, 2001). Thus, the trigeminal system detects tactile and/or noxious stimuli. In fact, the trigeminal chemosensory system consists of polymodal nociceptive neurons, where the associated endings are typically activated (most of the time, but not all of the time) by chemical irritants (Purves et al., 2004). The olfactory

nerve (cranial nerve I), on the other hand, only innervates the nasal cavity and can detect a wide range of odorant molecules.

Categorizations of trigeminal odorants have been based on the extent to which anosmics (individuals that lack a functional olfactory system) can either detect or describe these odorants (Doty et al., 1978; Cometto-Muñiz et. al., 1998, 2005). Previous studies have tested pure trigeminal chemical odorants by presenting them to the nostrils via an orthonasal route for smelling (Doty et al., 1978; Cometto-Muñiz et. al., 2005; Pierce and Halpern, 1996; Lundström, 2005,). Various vapor-phase trigeminal odorants have also been presented via a retronasal route (Voirol and Daget, 1986; Halpern 2004a, b; Heilmann and Hummel, 2004). Nontrigeminal odorants have also been subjected to retronasal vs. oral-cavity-only (OCO) testing (Chen and Halpern, 2006; Dragich and Halpern, 2006). Specifically, Dragich and Halpern (2006) found that if air-phase anise, cinnamon, coffee, orange, peppermint, and strawberry extracts were restricted to the OCO only peppermint natural extract was identified OCO. In fact, the major component of peppermint is menthol, which is a known trigeminal stimulus (Doty et al., 1978).

The present study focuses on various known trigeminal pure chemical odorants in the vapor-phase, comparing correct identifications (ID) for retronasal and OCO stimulation. There are many chemicals that are known trigeminal stimulants. Menthol (peppermint), allyl isothiocyanate (mustard, mustard oil), capsaicin (hot chile powder, mace spray) and diallyl sulfide (onion) are some known trigeminal chemicals. Statically, about 70% of all odors are said to stimulate the trigeminal nerve (Ohloff, 1994). However, not all of the chemicals are as effective trigeminally as they are olfactorially, and not all chemicals are equally effective olfactorially. Thus, each individual odorant

has varying degrees of sensitivity in both sensory systems. This study examined six known trigeminal chemicals: eugenol (CAS #97-53-0), heptyl alcohol (CAS #111-70-6), DL-menthol (CAS #89-78-1), nonanal (CAS #124-19-6), 1-octanol (CAS #111-87-5), valeric acid (CAS #109-52-4). From previous studies of these chemicals, eugenol is known to be the major component of clove odor and is used in perfumes and dental care products (Doty et al., 1978; Cometto-Muñiz et. al., 2005). Heptyl alcohol is characterized as a fragrant, woody odor (Doty et al., 1978; Laing et al., 2003). Nonanal has been described as floral-or citrus and 1-octanal as fatty-or waxy (Mahagan et al., 2004; Cometto-Muñiz et. al., 2005). DL-menthol is known to be the major component of peppermint odor (Doty et al., 1978; Murphy, 1983), and, lastly, valeric acid is characterized as having a sweaty, rancid odor (Doty et al., 1978; Brauchli et al., 1995; Chen and Dalton, 2005). Presenting the chemicals in the vapor-phase retronasally will simulate both the olfactory and trigeminal systems. However, restricting the odorant presentation to the OCO should stimulate the trigeminal system and not the olfactory system.

This study investigates if subjects could correctly identify the six vapor-phase stimuli when presented oral-cavity-only using the same identifications that subjects give for such stimuli when presented retronasally. We also wanted to see if there were any differences in reaction time to detect the stimuli when presented retronasally or OCO. Thus, the experiment design was to compare identifications of these six pure chemicals presented as vapor phase odorants retronasally versus OCO.

METHODS:

Subjects

Subjects were recruited with the use of online postings (www.susan.psych.cornell.edu), flyers, and word of mouth. Subjects were paid $6 US dollars for their participation in one session (<40 minutes). Participation was limited to non-smoking, non-pregnant, non-lactating individuals over the age of 18 who could communicate in American English. Subjects were asked to not eat or drink anything (except water) for at least one hour prior to each session. Sessions ranged from 20-40 minutes. Twenty subjects (ages 18 to 35, 9 females) were tested in the experiment to compare trigeminal (oral cavity only) and retronasal olfaction. Each subject was tested individually. The protocol was reviewed and approved by the Cornell University Committee on Human Subjects. Each subject read and signed an approved Informed Consent form before participating in the experiment. If the subjects had questions, they were answered by the experimenter as long as the answer would not prejudice the subject's response. The age and gender of the subject was noted in the experimenter's notebook.

Odorant delivery containers

The six odorants were presented using closed containers equipped with one straw (Odorant Delivery Containers) through which the vapor-phase odorants were delivered to subjects. The Odorant Delivery Containers (ODC) used in this experiment were 118 ml (4oz) black, oval, Ellipso Portion Cups with clear oval lids, manufactured by Newspring® Packaging and purchased from www.instawares.com (Chen and Halpern,

2006) (Figure 1). The Portion Cups and lids were made of homopolymer polypropylene. Two 5 mm diameter holes were made in the container lids; each hole was about 3.5 cm from the long ends of the Ellipso container lids, along the perimeter of an existing, large, circular imprint on the major axis of the lid. The distance from the center of one hole to the other was approximately 1.8 cm. The Ellipso Portion Cups and lids were washed in a glassware washer (Castle Model 7504, Sybron corporation) with soap (Alconox: powdered precision cleaner, VWR International, Westchester, PA 19380) and left to air-dry. A set amount of liquid or solid phase odorant (Table 1) was deposited to the bottom of a ODC. One homopolymer polypropylene Jetware® 7.75″ Unwrapped Plastic drinking straw (Jet Plastica Industries, Inc., 1100 Schwab Road, Hatfield PA 1440. (215)362-1501) precut to 6.5 cm was inserted into one of the holes in the ODC lid (hole not specified) so that half the straw (approximately 3.25 cm) was visible on each side of the lid. The straw had a diameter of 4.8mm. The lid with the straw was then closed onto the ODC cup. The straw was adjusted to stay vertical and taped with Scotch® tape to the lid. The black, oval cups were oriented with the bottom down and the lid on top when used as ODCs. Aluminum foil rectangles, with holes corresponding to the two holes in the lids, were positioned over the lids of the ODCs in order to prevent visual observation by subjects of the odorants.

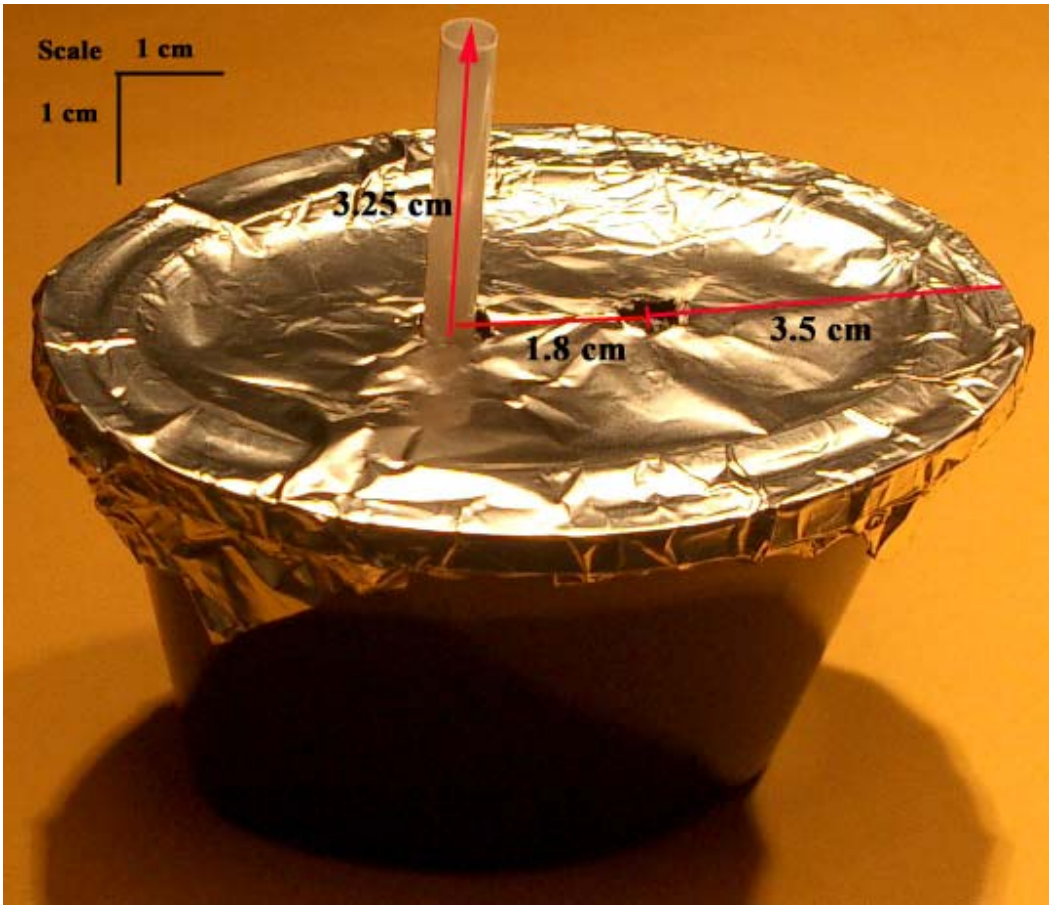

Figure 1. ODC container with straw and aluminum foil. Measurements are also included, with calibration line.

Odorants

Table 1 presents the odorants used, the amount of each odorant in the ODC, the correct identification of each odorant, and the numbers associated with the identifications, which were selected from a display on a digital computer to indicate each identification (see below). All odorants were used undiluted, i.e. neat. Concentrations and correct identifications of the odorants were based on previous reports (Doty et al., 1978; Murphy, 1983; Brauchli et al., 1995; Laing et al., 2003; Halpern 2004a, b; Mahagan et al., 2004; Chen and Dalton, 2005; Cometto-Muñiz et. al., 2005; Chen and Halpern, 2006; Dragich and Halpern 2006), as well as from benchmark studies and surveys given to people (n=34) around the lab prior to experimentation. All the responses given by those surveyed

were tabulated and the most frequent answer given that fit with previous reports for each chemical was used. All pure chemicals were purchased from Sigma-Aldrich Co (St. Lewis, MO). All subjects were presented with the ODC and their contained odorants at room temperature 21 ± 1 °C.

Table 1. The six odorants used, with the amounts in the ODC and correct identification.

Odorant	**Amount ml or grams**	**Correct Identification (number association)**
Eugenol (CAS# 97-53-0)	5ml	Cloves (1), Spice (2)
Heptyl alcohol (CAS# 111-70-6)	4ml	Cleaner (3)
Nonanal (CAS# 124-19-6)	0.3ml	Floral (4), Citrus (5)
1-Octanal (CAS# 111-87-5)	4ml	Citrus (5), Cleaner (3)
dl-Menthol (CAS# 89-78-1)	0.15 grams	Peppermint (6), ointment (7)
Valeric Acid (CAS# 109-52-4)	3ml	Rancid (8), Sweat (9)

A random order of pure chemicals to be presented was accomplished as follows: Chemicals were written down in alphabetical order and then assigned the numbers 1-6 alphabetically. A page with a table of random digits (Table o. Ten-Thousand random digits) from "Statistical Tables" was selected. The numbers 1 through 6 were recorded vertically and down the page from the start location determined by blindly pointing to a spot on the page with the pointed end of a pencil. Repeated and unassigned numbers

were discarded. Six unique orders were generated in this way. The orders were written in a lab notebook. Three sets of unique orders were used for retronasal testing and three unique sets were used for trigeminal testing.

SuperLab Program

Stimulus presentation software, SuperLab© 4.0 (Cedrus Corporation**,** P.O. Box 6309, San Pedro, CA 90734), was used to present slides of instructions and choices of possible odor identifications and their number associations for the six pure chemicals to the subject. The program was also used to record responses that subjects made (see below) as well as input from the microphone set up (see below) to The SuperLab program provided feedback by advancing to the next side on the when subjects exhaled via either their nose or mouth after inhaling the odorants.

Microphone Set up

Subjects were asked to wear a unidirectional hands-free Headset microphone with a 100-12000Hz frequency response range (Radio Shack part # 33-3012) over the temple of one side of their face around the back of the head and over their temple of their other side of their face (Figure 2). Protruding out of the subjects face was a receiver, which contained the unidirectional microphone. The headset's 1/8 inch (3.5-mm) plug was connected to a MaCally Ivoice USB 2.0 microphone adaptor (UPC No. 701107483528) which was connected to a free USB 2.0 slot in an iMac PowerMac 4.2 running OS X 10.4.9 (8P135). The unidirectional receiver of the microphone was placed one inch away from the mouth when conducting trigeminal trials and one inch away from the nose and once inch away from the mouth when conduction retronasal trials (Figure 2). Once the

subjects had the head set in place, they were asked to breathe in and out of their nose normally in order to set the a threshold level of the output volume of the microphone during retronasal testing. Once this threshold volume level was reached, the "exhalation slide" in the SuperLab script advanced to a slide showing choices of possible odor identifications and their number associations for the six pure chemicals or the "choices slide" (see ***Retronasal Odor Identification Test Procedure***). A new threshold was set for the detection of exhalation via the mouth for the OCO trials. Each subject required a different threshold level. After each subject finished the study, the Large Acoustic-Foam Microphone Windscreen (Radio Shack part # 33-4001), which is a foam piece that fits snugly over the microphone head to help reduce wind and breathing noise, was removed and irradiated with a UV light (General Electric Reflector Sunlamp Kit, Model RSK5, General Electric company, Nela Park, Cleveland, OH. 44112). The piece was placed four inches away from the UV light for 10 minutes in order to sterilize it.

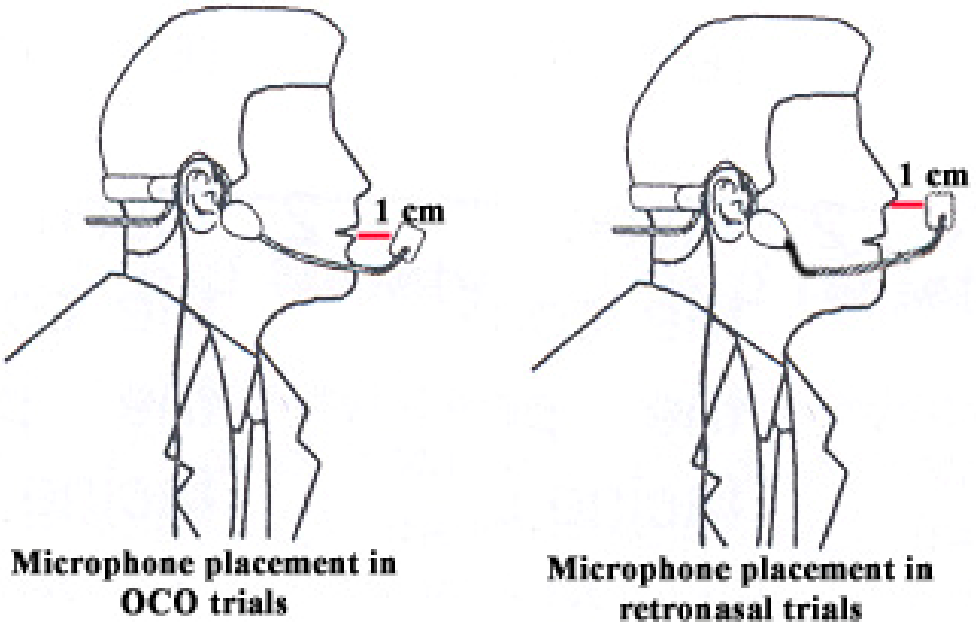

Figure 2. Microphone set up. The headset is placed over the ears and the microphone is 1 cm away from the mouth in retronasal trials and 1 cm away from the nose in OCO trials.

Subject Training Procedure

The subject was told that the experiment compares two different methods of sensing odors, retronasally and trigeminally. Retronasal olfaction was explained as inhaling through your mouth and exhaling through your nose. Trigeminal perception was explained as inhaling through your mouth and exhaling through your mouth and was referred to as "Oral Smelling". The subject was presented with written instructions on the computer screen before testing. After reading the instructions thoroughly, the retronasal and trigeminal procedures were verbally explained and demonstrated by the researcher using an empty ODC.

The subject was presented with a list of nine possible odor identifications and their number associations for the six pure chemicals. Retronasal and trigeminal presentation of the odors were used to familiarize the subjects with the odors and names. ODC with aluminum foil on top, as previously described, were used to avoid correct identifications (ID) based on the visual appearance of the odorant. The subjects were given ODCs one at a time and were told what the correct ID of each ODC was. For example, the subject was given an ODC that contained eugenol and was told that the correct ID of this ODC was cloves or spice. The subjects were then instructed to acquaint themselves with the odors well enough to be able to identify them again later. They were given as much time as the wanted to connect stimuli with odor name.

Subjects went through a test trial on the computer (iMac PowerMac 4.2 running OS X 10.4.9 (8P135) with a 15 inch LCD 1024 x 768 resolution display) and were asked to identify the six odorants first retronasally, and then trigminally in a randomly generated order. The first slide of the computer program, SuperLab 4.0, presented the

subjects with a list of the six possible identifications and their number associations for the six odorants. Subjects were seated, facing the computer display. The viewing distance between the subject and the display screen was 20 inches. Subjects were told that the odorant names displayed on the first SuperLab slide were the odorants they had just acquainted themselves with.

After completing the test trial, the subjects were asked if they had any questions about the computer set up. Any questions about the computer set-up were answered. If the subject had any questions about correct odor identification, the subject was told to pick the best identification possible.

Procedures for both retronasal and trigeminal testing:

A flow chart of the full procedure is shown in table 2.

Table 2. A flow chart of the experimental procedure.

Retronasal	Trigeminal
1. Informed consent form was singed and questions answered. 2. Set up microphone for appropriate retronasal threshold level. 3. Subject training/familiarization of odorants. 4. Subject training/familiarization of retronasal smelling with ODCs and computer set up. 5. Retronasal odor identification test (x3) a. First ODC given for identification b. 30 second wait c. Second ODC given for identification d. 30 second wait e. Third ODC given for identification f. 30 second wait g. Fourth ODC given for identification h. 30 second wait i. Firth ODC given for identification j. 30 second wait k. Sixth ODC given for identification	1. Set up microphone for appropriate trigeminal threshold level. 2. Subject re-training/re-familiarization of odorants. 3. Subject training/familiarization of trigeminal smelling with ODCs and computer set up. 4. Trigeminal odor identification test (x3) a. First ODC given for identification b. 30 second wait c. Second ODC given for identification d. 30 second wait e. Third ODC given for identification f. 30 second wait g. Fourth ODC given for identification h. 30 second wait i. Firth ODC given for identification j. 30 second wait k. Sixth ODC given for identification
Note: The order of which chemical to present for each set of an odor identification test was randomly selected as explained prior. Each set also had a different random order of chemical presentation.	

There were 36 odorant presentations. Each odorant was blocked in groups of 6, i.e. each of the six had to occur once before the next occurrence of that odorant. Thus, one block had six odorants presented in a randomized order. The order of the group of 6 odorants was also different in different blocks. There were three blocks (18 odorant presentations) presented in the retronasal testing and three blocks (18 odorant presentations) presented in the trigeminal testing.

Retronasal and trigeminal trials were randomized. The subjects were asked to identify the odor retronasally and trigeminally using the method described in the odor identification test. The six chemicals were presented, and the subject was asked to pick the odor identification off the list that best identified the odorant sensed. The subject would get one chance to identify the odor, if an incorrect response was made, there was no correction and the experiment continued. If no response was recorded after ten seconds, the next presentation would be administered. Subjects who accidentally pressed the wrong key were asked to indicate which key they intended to press. This corrected ID was written, along with other errors in the researcher's lab notebook. The intended key was used in data analyses. Subjects were told not to press anything if they did not detect anything. If the subjects were uncertain about any odor, they were allowed to re-familiarize themselves with the odors that they could not remember. Then the computer portion of the experiment began.

Retronasal Odor Identification Test Procedure:

The subject was given time to read through the Instructions for Retronasal Olfaction slide (Figure 3) After allowing the subject to read through the instructions on

the SuperLab script, he/she attached a nose clip (Spirometrics Nose Clip #2104, Spirometrics, P.O. Box 680, 22 Shaker Rd. Gray, ME 04039; (207)657-6700) to their nose and exhaled through their mouth. After exhaling, the subject was asked to press the space bar. Prior to pressing the space, if the subject exhaled above the set threshold and produced microphone input, nothing happened. That is, until the space bar was pressed by the subject during the "nose clip/exhalation/spacebar" instructions slide (Figure 4), the SuperLab script did not recognize the microphone input. However, once the subject pressed the space bar, if the subject exhaled above the set threshold, the SupereLab script was activated for microphone input and marked the time of exhalation. An ODC was given to the subject by the experimenter. The subject was instructed to place his/her mouth over the straw, close their mouth and make a seal around the straw and inhale through the mouth, remove the nose clip, and exhale through his or her nose (Figure 5). This exhalation, detected by the microphone, caused the SuperLab slide to advance to the next slide containing a list of the nine possible odorants (Figure 6). The subject could then press the number on the keyboard using either the numeric keypad or the number row of the keyboard that corresponded to the odor identified (Figure 6). If they could not identify the odorant, the subject was told not to press anything. The SuperLab script was set to record the subjects choice and the time that elapsed from exhalation to key press. The subject was asked to wait for 30 seconds before inhaling the next odorant.

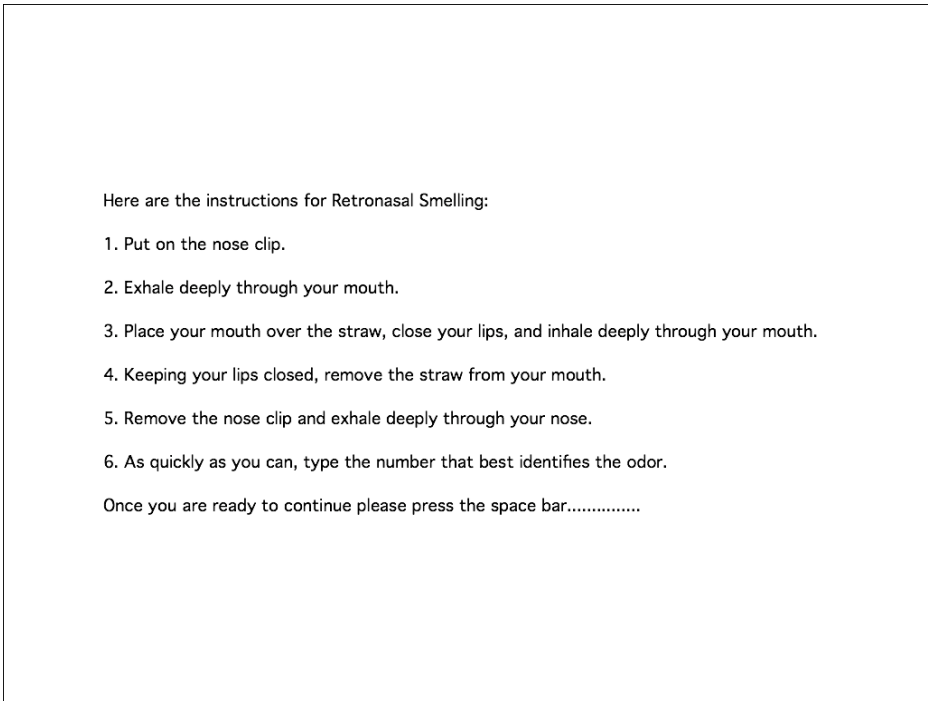

Figure 3. Retronasal SuperLab script #1 - Instructions for retronasal smelling. (“ Retro Instruction Slide”). Note: microphone inactive in this slide.

Figure 4. Retronasal slide #2 (“Nose clip slide”) Note: microphone inactive in this slide.

Figure 5. Retronasal slide #3 (“inhalation/exhalation slide”). Note: microphone activated in this slide.

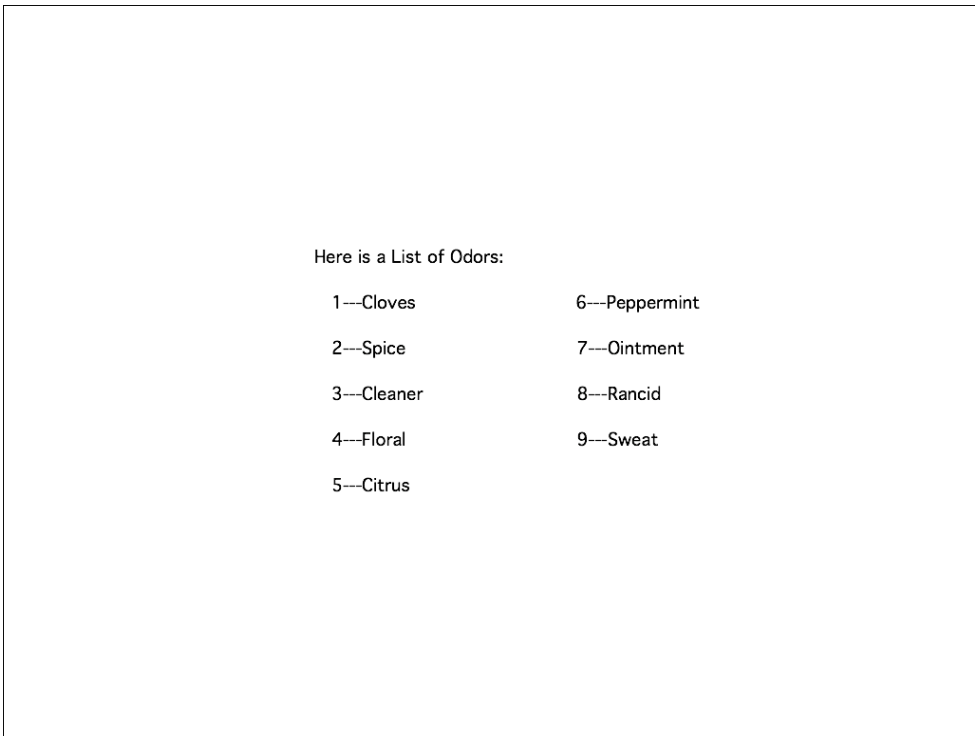

Figure 6. Retronasal slide #4 (“choices slide”). Note microphone inactive in this slide.

Trigeminal Odor Identification Test Procedure:

The procedure for trigeminal odor identification was the same as the procedure for retronasal odor identification, with one modification. That is, when the subject was presented with the ODC, the subject was instructed to place his/her mouth over the straw, close their mouth and make a seal around the straw and inhale through the mouth, and at their own pace exhale through his or her mouth, keeping the nose clip on his or her nose (Figure 9). This being different than the retronasal procedure in that subjects kept their nose clip on their nose and they exhaled through their mouth, not their nose. The slides for the trigeminal odor identification test are shown in Figures 7-10.

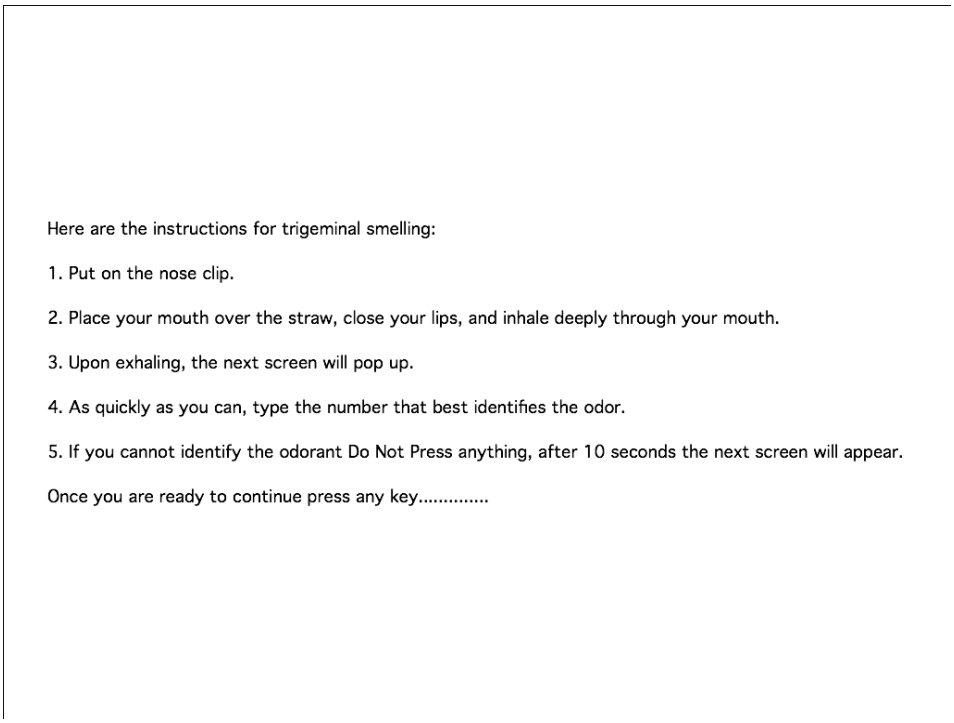

Figure 7. OCO SuperLab script #1 - Instructions for retronasal smelling. ("OCO Instruction Slide"). Note: microphone inactive in this slide.

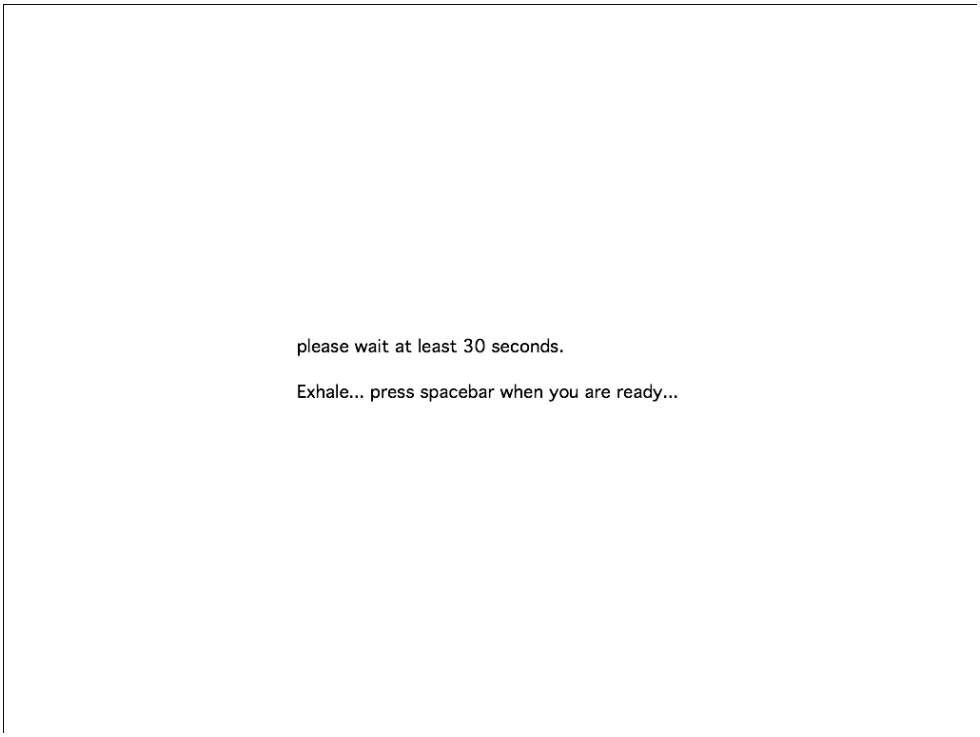

Figure 8. OCO slide #2 (“Nose clip slide”) Note: microphone inactive in this slide.

Figure 9. OCO slide #3 (“inhalation/exhalation slide”). Note: microphone activated in this slide.

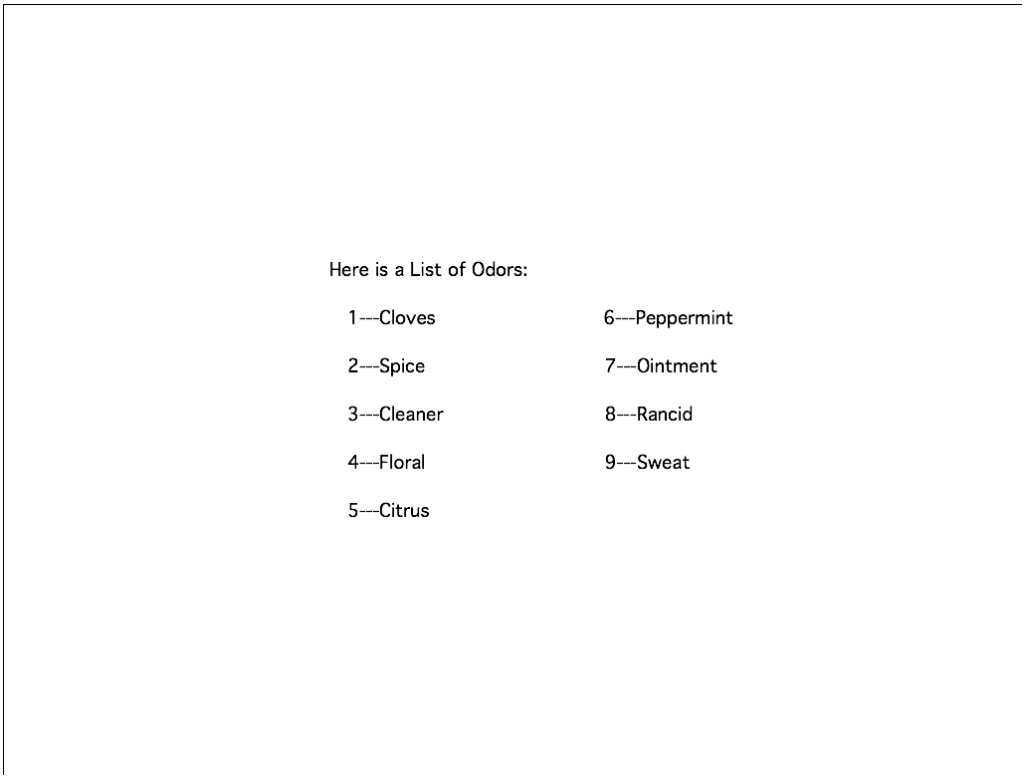

Figure 10. OCO slide #4 ("choices slide"). Note microphone inactive in this slide

Statistical Analysis

Non-parametric statistics were chosen for analysis because of the relatively small sample size of this study and in order to avoid unnecessary the assumptions, such as that of a normal distribution of the sample. An α level was set to 0.05. Correct ID of the odorants were obtained by assigning a value of 0 for no response or incorrect response and a value of 1 was assigned for correct identification. Medians of the three IDs for each odorant for each subject under the two presentation conditions were used to calculate percent correct IDs and semi-interquartile ranges (SIR) (Tables 3), and for inferential statistics. For comparisons of the odorants presented retronasally and OCO, Freidman non-parametric ANOVA tests were used. For pairwise comparisons of each odorant retronasally vs. OCO, Wilcoxon signed rank tests were used.

RESULTS:

Identifications

Overall, for eugenol, heptyl alcohol, nonanal, 1-octanal and valeric acid, the percent of correct IDs was higher retronasally vs. OCO (Table 3). For retronasal smelling of those five odorants, overall percent correct ID ranged between 49.2% to 79.7%; for OCO, overall percent correct ID ranged between 5% to 20%. Dl-menthol also showed a higher percent of correct ID retronasally vs. OCO (79.7% vs. 58.3% respectfully), however, the overall percent of correct ID OCO was greater than any other odorant presented OCO and was greater then heptyl alcohol and nonanal presented retronasally. Median percent correct ID retronasally ranged from 100% for eugenol and dl-menthol (SIR from 27.1% to 20.8%) to 66.7% for heptyl alcohol, 1-octanal and valeric acid (SIR from 50% to 16.7%) and 58.3% for nonanal (SIR = 33.3%). For OCO, median percent correct ID was 0% for all odorants other than dl-menthol (SIR from 16.7% to 0%). For dl-menthol, median percent correct ID was 66.7% (SIR = 33.3%) (Table 3).

For the 6 odorants presented retronasally and OCO, there was a significant difference in the number of correct IDs given by the subjects ($p < 0.0001$, $df = 12$, chi square = 118.920, Friedman Non-Parametric ANOVA). Across odorants presented retronasally, there was a significant difference in number of correct ID ($p = 0.0347$, $df = 5$, ch-square = 17.921), and across odorants presented OCO, there was a significant difference in number of correct ID ($p < 0.0001$, chi-square = 41.236, $df = 5$) Friedman Non-Parametric ANOVA. A pairwise comparison of the number of correct IDs given by subjects for dl-menthol presented retronasally vs. OCO showed no significant difference ($p = 0.1026$, $Z = -1.632$, Wilcoxon Signed Rank Test). However, pairwise comparisons

for the other 5 odorants (other than dl-menthol) presented retronasally vs. OCO found that the number of correct ID was significantly greater when presented retronasally ($p \leq 0.0029$, $Z < -2.982$, Wilcoxon Signed Rank Test).

The confusion matrix (see Kurtz et al., 2001; Sun and Halpern, 2005; Wright, 1982) component of Table 3 (see PERCENT OF EACH ID SELECTED FOR EACH ODORANT AND PRESENTATION CONDITION) indicated that retronasally, nonanal was mistaken for cleaner 18.6 % of the time, valeric acid was mistaken for citrus 11.9% of the time and heptyl alcohol was mistaken for citrus 11.0% of the time and rancid for 10.2% of the time. Similarly, in OCO, valeric acid was also mistaken for citrus 15% of the time, heptyl alcohol was mistaken for rancid 13.3% of the time and nonanal had 10% incorrect identification for both cleaner and rancid.

Table 3. Overall % correct identifications (IDs), median % correct IDs and SIR, and % of each ID selected for each of 9 IDs (plus no re

Each of six odorants three times, randomized in blocks of six, either retronasally or oral-cavity-only. Underlined, boldface % are correc

			PERCENT OF EACH ID SELECTED FOR EACH ODORANT AND PRESENTATIO							
ODORANT	Overall % correct IDs	Median% correct IDs (SIR)	No response	Cloves	Spice	Cleaner	Floral	Citrus	Pepperment	Oi
Eugenol						Retronasal				
	78.0%	100% (27.1%)	0.0%	**49.2%**	**28.8%**	0.0%	5.1%	1.7%	5.1%	
						Oral-Cavity-Only				
	6.7%	0%(0%)	88.3%	**6.7%**	**0.0%**	1.7%	0.0%	0.0%	1.7%	
Heptyl alcohol						Retronasal				
	49.2%	66.7% (50%)	3.4%	1.7%	1.7%	**49.2%**	8.5%	11.9%	1.7%	1
						Oral-Cavity-Only				
	5.0%	0%(0%)	65.0%	3.3%	6.7%	**5.0%**	0.0%	5.0%	1.7%	
Nonanal						Retronasal				
	54.2%	58.3%(33.3%)	0.0%	1.7%	8.5%	18.6%	**20.3%**	**33.9%**	1.7%	
						Oral-Cavity-Only				
	10.0%	0%(0%)	50.0%	5.0%	8.3%	10.0%	**1.7%**	**8.3%**	3.3%	
1-Octanal						Retronasal				
	71.2%	66.7%(16.7%)	3.4%	0.0%	3.4%	**54.2%**	6.8%	**16.9%**	3.4%	
						Oral-Cavity-Only				
	18.3%	0%(16.7%)	56.7%	5.0%	6.7%	**10.0%**	3.3%	**8.3%**	5.0%	
dl-menthol						Retronasal				
	79.7%	100%(20.8%)	1.7%	6.8%	6.8%	0.0%	5.1%	0.0%	**57.6%**	**2**
						Oral-Cavity-Only				
	58.3%	66.7%(33.3%)	31.7%	5.0%	0.0%	5.0%	0.0%	0.0%	**46.7%**	**1**
Valeric Acid						Retronasal				
	66.1%	66.7%(33.3%)	1.7%	5.1%	3.4%	1.7%	1.7%	11.9%	5.1%	
						Oral-Cavity-Only				
	20.0%	0%(16.7%)	43.3%	5.0%	3.3%	5.0%	3.3%	15.0%	5.0%	

SIR = Semi-interquartile range, i.e., the difference resulting from the first quartile (Q1) subtractred from the third quartile (Q3), divided
Median, Q1 and Q3 were calculated from the number of IDs by each of 20 subjects for correct IDs for an odorant.
Note, there was not a time when the subjects indicated that he/she pressed an incorrect letter by mistake. The intended key was thus pres

Reaction Time:

Overall, the median reaction time was less for the odorant presented retronasally then compared to odorants presented OCO for five odorants expect for dl-menthol (Table 4). For dl-menthol, the median reaction time was slightly less when the odorant was presented OCO than retronasally. A pairwise comparison of the reaction given by subjects for all the odorants presented retronasally vs. OCO showed no significant difference ($p \geq 0.1771$, $Z \leq -0.365$, Wilcoxon Signed Rank Test).

Table 4. Overall median ID reaction times, and SIR, in seconds, for the six odorants presented to 20 subjects, both retronasally and oral-cavity-only.

Odorant	Presentation	Median Reaction Time (sec)	Time SIR (sec)
Eugenol	Retronasal	3.43	0.94
	OCO	4.12	1.3
Heptyl Alchoal	Retronasal	4.69	0.72
	OCO	4.89	1.22
Nonanal	Retronasal	4.5	0.92
	OCO	5.13	1.56
1-octonal	Retronasal	4.38	0.856
	OCO	5.93	1.3
dl-menthol	Retronasal	3.69	0.82
	OCO	3.36	1.13
Valeric Acid	Retronasal	3.96	1.27
	OCO	5.23	1.01

SIR = Semi-interquartile range, i.e., the difference resulting from the first quartile (Q1) subtractred from the third quartile (Q3), divided by 2 [(Q3 - Q1)/2]

Median, Q1 and Q3 were calculated from the number of IDs by each of 20 subjects for correct IDs for an odorant.

DISCUSSION:

Dl-menthol was the only odorant which, when presented OCO, had the same or higher median percent correct ID (66.7%) as four other odorants presented retronasally (heptyl alcohol, nonanal, octonal and valeric acid). The median correct ID, however was not was not as high as dl-menthol and eugenol presented retronasally (100% correct ID). Pairwise comparisons of dl-menthol presented retronasally vs. OCO did not show any statistical significance. It should be noted that the overall percent correct ID is somewhat greater for dl-menthol retronasally (79.7%) than OCO; however some other odorants presented retronasally had lower percent correct ID (49.2% for heptyl alchoal, and 54.2% for nonanal) then the median percent correct ID of dl-menthol presented OCO (58.3%). Even when comparing the odorants presented only via OCO using inferential non-parametric statistics, percent correct ID of dl-menthol was significantly higher than any of the other five odorants. Consequently, of the six trigeminal vapor-phase stimuli tested, only dl-menthol, when presented OCO, had a percent correct ID comparable to the retronasal percent correct ID. Thus, subjects could identify dl-menthol when presented retronasally about the same as when presented OCO. This suggests that oral trigeminal responses to dl-menthol have sufficient differential information to not only permit discrimination from other trigeminal stimuli but also to allow ID comparable to retronasal ID.

One factor that might skew the results is that subjects are more familiar with dl-menthol, a major component of peppermint. The median percent correct ID of dl-menthol retronasnally is 100%. However, so is the median percent correct ID of eugenol. So from

the results, subjects equally identified and are equally familiar with dl-menthol and eugenol when presented retronasally. In fact, the overall percent correct IDs of both odorants retronasally are also very close together (79.7% for dl-menthol and 78.0% for eugenol). When presented OCO, subjects could hardly if at all identify eugenol. If it was the case that subjects are more familiar with the smell of dl-menthol, then the median and overall percent correct ID of eugenol when presented OCO should be similar to that of dl-menthol when presented OCO. The results indicate that this is not the case.

There are a number of hot and cold transient receptor potential (TRP) ion receptors in the mouth that are expressed in the neurons of trigeminal and dorsal root ganglia (McKemy et al, 2002; Peier et al., 2002). Specifically, McKemy et al. and Peier et al. found that these new channels open to mildly cold temperatures and to menthol. Thus, with the induction of dl-menthol into the mouth, subjects TRP receptors opened and they perceived a cooling feeling. Subjects could have associated this feeling with that of ointment or peppermint to describe the cooling feeling. There have no other specific receptors associated with the other five stimuli.

For eugenol, heptyl alcohol, nonanal, octonal and valeric acid, some correct ID were selected in the OCO presentation. Since OCO restriction was achieved by a presence of a nose clip that prevented exhalation to the nostrils, there could have been some access to the nostrils by way of diffusion of the odorants from the oral cavity to the nostrils. However, since reaction time did not significantly differ between OCO presentation and retronasal presentation, this assumption does not seem correct.

The overall percentage of no responses to odorants when the stimuli were presented OCO ranged from 88.7% to 31.7%. Eugenol had the highest percent of no

response and dl-menthol had the lowest. The other four odorants had no responses between 65% and 43.3%. Subjects stated a no response to valeric acid when presented OCO 43.3% of the time and the correct response was only give 20% of the time. Thus, 23.3% of the time, subjects did notice that there was an odorant in their mouth; however they could not identify it. With this, it seems as though it is important to not only look at the percentage of correct ID, but also to look at the percentage of no response to an odorant when judging if subjects could identify such stimuli when restricted to the OCO. Subjects might feel there is another identifier that describes what they are feeling in the OCO that was not on the list of identifiers. This is one limitation of this study. The identifiers given for the six odorants are very subjective. Some subjects could have also forgotten what the correct ID was for an odorant as the experiment went along even though they were given a chance to re-identify themselves with the odorant whenever they wanted in the experiment. No subjects ever took advantage of this. They might have been too shy or embarrassed to say they forgot what the correct ID was for a certain odorant. This has implications to this study in that there would be less percentage of correct ID of an odorant. Future studies could have a mandatory re-familiarization stage of odorants after say a certain amount of time.

The main outcome of this study is the inability of subjects to select for the correct OCO ID for five of the six known trigeminal stimuli. This is somewhat surprising in that all the six odorants are known trigeminal stimuli. Even if the odorants did stimulate the trigeminal system, the inability of subjects to identify all the odorants expect dl-menthol when presented to the OCO suggests that subjects cannot correctly identify the five odorants with descriptors used for both retronasal and orthonasal smelling. This suggests

that future work needs to be done with the five other odorants using other identifiers to see if there are any other descriptors that subjects can better identify with them the odorants are presented OCO. If using other identifiers fails, then it could very well be the case that subjects cannot use the same descriptors used when presenting the odorants retronasally or orthonasally.

Future studies can also look at the biochemistry of dl-menthol to explain why it is such a strong trigeminal stimulus. Investigating the properties of dl-menthol, with respect to chemistry and shape to see what makes it such a strong stimulus could also be another direction in future research. Comparing dl-menthol with the properties and characteristics of the other five odorants chemically might give a better insight as to what's different about dl-menthol. Lastly, one can use the other isomeric forms, such as the dextrorotary form, or levorotary form only, of dl-menthol and present them to subjects to ascertain whether or not there is a difference in the correct ID.

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