

Orthonasal and retronasal odorant identification based upon vapor phase input from  
common substances

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Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

**ABSTRACT**

Subjects were trained to identify by assigned number common substances presented as vapor phase stimuli via an orthonasal or a retronasal route. Following training, odorant identification learning was evaluated by measuring ability to correctly identify to a criterion. Those who met the criterion were then tested 1st with the stimuli presented to the nares that differed in location from the nares used in training, and 2nd, to the nares that corresponded in location to the nares used in training. It was found that, under conditions of natural retronasal breathing, orthonasally trained subjects, upon retronasal testing, made correct identifications on approximately 80% of the trials, but for the following orthonasal testing, identifications were significantly more frequent, approaching 100% correct. After subsequent retronasal training, the same subjects' orthonasal identifications remained significantly higher, although identifications improved to approximately 92% correct on retronasal trials. Other subjects were instructed in a breathing technique designed to enhance retronasal stimulation. After orthonasal training, retronasal testing of these subjects still gave significantly fewer correct identifications than orthonasal testing, notwithstanding the modified retronasal breathing, but after subsequent retronasal training, correct identifications by these subjects no longer differed significantly between orthonasal and retronasal testing. Efficacy of modified retronasal breathing was confirmed in two subsequent experiments. The observed substantial positive transfers between retronasal and orthonasal odorant identification training and testing loci demonstrate that these odorant pathways do not subservise completely independent olfactory systems, while the less accurate identifications via the retronasal route, unless instruction in retronasal breathing was given, suggests a difference in the efficiency with which odorants are normally delivered to the olfactory mucosa.

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

**Introduction**

According to Cain (1987), there exist "two modalities that 'stand guard' over what we eat." These are taste and olfaction. Cain asserts that smell presents the more complex problem; numerous unresolved questions remain. An intriguing aspect of olfaction is the existence of two distinct pathways through which stimuli can arrive at the olfactory epithelium. Orthonasal stimulus delivery occurs when odorants travel inward from the anterior nares (nostrils) towards the olfactory mucosa, while retronasal stimulation is caused by the ascent of odorants through the posterior nares of the nasopharynx (DeWeese and Saunders, 1968; Davis, 1980; Voirol and Daget, 1986; Roberts and Acree, 1995). Most typically, orthonasal olfaction occurs during respiratory inhalation or sniffing; retronasal olfaction, during respiratory exhalation or after swallowing.

Orthonasal and retronasal olfaction might be quite separate and functionally different olfactory systems, or instead, the odorant pathways that begin with the anterior or posterior nares could simply be alternative routes to a shared olfactory receptor surface and neural apparatus. These contrasting possibilities can be subjected to empirical tests. The degree of independence of retronasal and orthonasal olfactory perception, the extent to which any non-independence is mutually shared by orthonasal and retronasal pathways, and the potential reasons for asymmetry, are studied in the present experiments.

An alternative use of the term 'retronasal' combines intraoral liquid stimulation, potentially both taste and oral trigeminal, with odorant input via the posterior nares (e.g., Kuo, Pangborn, and Noble, 1993). This usage is perhaps captured by the term 'oral' (Burdach, Kroeze, and Koster, 1984). However, although the intraoral presence of a liquid that provides potential gustatory, olfactory, and trigeminal stimuli may approximate the condition that commonly occurs after the drinking of liquids or during mastication of foods (Gibson, 1966; Roberts and Acree, 1995), this array of stimuli also precludes studying any one sensory system in isolation. An analysis directed towards odorant input through the

**Orthonasal and retronasal odorant identification**  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

posterior nares requires that direct gustatory and trigeminal stimulation of the tongue, at least, be excluded, while an interest in relatively constant intraoral vapor phase stimulation necessitates that interactions between odorants and saliva, and effects of intraoral temperature, be minimized (Roberts and Acree, 1995). The experiments to be presented in this report are designed to study responses dependent only upon vapor phase stimulation from odorants, and therefore utilize the more restrictive meaning of retronasal.

Quantitative comparisons of perceived intensity for human orthonasal olfaction versus human retronasal olfaction or retronasal-gustatory 'oral' stimulation have been done by a number of investigators (e.g., Murphy and Cain, 1980; Burdach et al., 1984; Voirol and Daget, 1986; Burdach and Doty, 1987; Cain, 1988; Kuo et al., 1993). Generally, these studies found greater intensity for orthonasal than for retronasal stimulation when only vapor phase stimuli were made available to the anterior or the posterior nares (e.g., Voirol and Daget, 1986). Comparisons of orthonasal and oral (retronasal and liquid-intraoral) stimulation have produced complex or conflicting results, with apparent compound-specific interactions, and instances of so-called cognitive associations between particular odorants and tastants (e.g., Murphy and Cain, 1980; Burdach et al., 1984; Kuo et al., 1993).

Investigations of odorant identification involving human retronasal olfaction have been less common. Rozin (1982) found that blindfolded subjects, after learning to identify by number the vapor phase component of four unfamiliar soups or juices delivered orthonasally, performed better on orthonasal recall tests than on oral stimulation (1.2 ml of liquid stimuli injected into the mouth) recall tests. Although the 58% to 66% correct numerical identifications on oral trials were better than a chance level of 25%, they were inferior to the mean of 83% correct identifications on orthonasal trials. From this superiority of orthonasal identifications, Rozin argued that there exists a qualitative difference in the perception of an odorant depending on whether it is perceived orthonasally

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

or retronasally. He called this phenomenon an "olfactory duality," offered several hypotheses in explanation, and suggested empirical tests of the hypotheses (Rozin, 1982).

A direct test of the degree of olfactory perceptual independence between retronasal and orthonasal routes would be to train subjects to identify odorants using retronasal vapor phase presentations, and then test identification accuracy for orthonasal vapor phase presentations. If the orthonasal and retronasal-gustatory 'oral' identification differences reported by Rozin (1982) were solely due to a synthesis or amalgamation of the retronasal odorant and lingual gustatory or trigeminal responses elicited by the mixtures of exotic soups or juices that were used as oral stimulus liquids, then retronasal and orthonasal differences would vanish if only vapor phase retronasal and orthonasal stimuli were used (e.g., Murphy and Cain, 1980; Gillan, 1983; Enns and Hornung, 1985). Finally, if the critical factor underlying the discrepancy between the retronasal and orthonasal responses is a difference in stimulus input to the olfactory mucosa, then modifications in odorant delivery, such as an increase in retronasal flow properties, might be capable of reducing or eliminating a dissimilarity in ability to identify odorants that Rozin (1982) observed under more natural conditions.

In order to unravel the influence of vapor phase versus oral liquid stimulation and the significance of the nature of retronasal vapor phase flow on odorant identification, the present investigation restudied the proposed "olfactory duality" (Rozin, 1982). The orthonasal and retronasal pathways of vapor phase stimulation were utilized under conditions of no known mouth movements, no oral manipulation of the odorant, and no known taste stimulation, in four experiments. According to Rozin's duality hypothesis, if the reverse of his experiment was performed, that is, retronasal identification training followed by orthonasal and retronasal recall tests, subjects should perform more poorly on the orthonasal tests than on the retronasal tests (Rozin, 1982). This prediction was directly tested in three of the experiments. Modifications of retronasal breathing were also

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

instituted. A brief report of the first three experiments has been made (Pierce and Halpern, 1995).

## **Methods**

### GENERAL PROCEDURES

#### Subjects:

The proposed research was submitted to, and approved by, the Cornell University Committee on Human Subjects. All subjects were non-pregnant unpaid volunteer Cornell University undergraduate students, at least 18 years of age. Subjects ages ranged from 18 to 24 years; mean = 20.8 years; median, 21 years.

#### Screening

Prior to the beginning of each main experiment, subjects were screened to ensure ability to respond to vapor phase stimuli. Five odorants previously used by Cain and Krause (1979) were employed. The screening stimuli were lemon juice (Borden's Lemon Juice), peanut butter (Tops Extra Creamy), mothballs (Enoz Old Fashioned Mothballs), Cherry KoolAid<sup>®</sup> (powder), and either minced Ivory Soap<sup>®</sup> (Experiment One) or Hershey's Unsweetened Baking Chocolate (Experiments Two through Four). Screening stimuli were presented in chemically clean, odorless, high density polyethylene, 5 cm high, 3 cm diameter open cylinders closed at one end.

Disposable latex plastic gloves were worn by the experimenters throughout screening procedures. Subjects were asked to close their eyes during screening stimuli presentations, and then sniffed all five odorant-containing cylinders for 10 s each while the stimuli were successively held below their nostrils. After the last cylinder was removed, subjects immediately looked at a list consisting of the names of the five screening stimuli

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

and ten others, namely: apples, bananas, cherries, chocolate, coffee, garlic, ginger, lemon juice, lime, mint, moth balls, oregano, peanut butter, paprika, and soap, and were tested for ability to respond to vapor phase stimuli by being asked to identify the presented odorants from the list. If unsure, subjects were permitted to repeat this sampling procedure. Only the first five selections from the list were accepted, but the order did not have to correspond to the sequence in which the screening stimuli were presented. Subjects needed to correctly recognize at least three of the five odorants to continue in an experiment. All subjects in all four experiments successfully met this screening criterion.

Main Experiment Odorants and Procedures

Odorants

The four odorants used in main experiments were Spice Islands Oregano Powder, Maxwell House Filterpack ground coffee, McCormick's Garlic Powder, and either Hershey's Unsweetened Baking Chocolate (Experiment One) or Ivory Soap<sup>®</sup> (Experiments Two through Four). Approximately 0.5 - 0.9 grams of these odorants were presented. The Hershey's Unsweetened Baking Chocolate or Ivory Soap<sup>®</sup> were minced into pieces no larger than 1 mm in diameter. The other odorants listed were purchased in sufficiently ground form to be used without further modification.

Odorant Presentation Containers and Techniques

The odorant presentation containers for all main experiments were the gray covers of Kodak Ektar<sup>®</sup> 1000 film canisters. These odorant presentation containers were two chemically clean, odorless, low density polyethylene concentric open cylinders, closed at the bottom with a common base. They were used because for retronasal stimulation, the outer diameter and overall height would permit comfortable placement inside the mouth, the inner cylinder would hold a sufficient volume of solid odorant, the separation between the inner and outer cylinders would prevent direct contact between the odorant and oral tissues, and

**Orthonasal and retronasal odorant identification**  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

the high density polyethylene composition would neither act as a gustatory stimulus nor interact with the odorants. The outer cylinder was 5.4 mm high with a 3.7 cm diameter and 1.3 mm wall thickness. The inner cylinder, within which odorants were placed, was 6.1 mm high with a 2.9 cm diameter and a 0.6 mm wall thickness; both cylinders had a 1.0 mm thick bottom. Disposable latex plastic gloves were worn by experimenters throughout orthonasal and retronasal presentations.

To prevent visual identification, subjects were asked to close their eyes during orthonasal and retronasal presentations. Observation and inquiry during each experiment confirmed that subjects complied with this instruction. All subjects' responses were communicated verbally, and recorded by the experimenters.

For orthonasal odorant presentation, an odorant presentation container was suspended by hand approximately 3 - 4 mm below the subject's anterior nares (nostrils). Subjects were told to breathe normally through their nose, with their mouth closed. Normal breathing was specified because no retronasal maneuver comparable to an orthonasal sniff was available. To roughly distinguish between normal breathing and sniffing, an approximately 1 cm<sup>2</sup> piece of facial tissue was taped to the tip of the nose, next to, but not blocking, the nostrils. Increases in inspiration air flow rate caused the tissue to flutter towards the nostrils. Subjects who accidentally sniffed were reminded to breathe normally. Orthonasal stimuli were presented this way for 10 s during odorant identification training (see below), while in testing sequences, subjects had up to 10 s to respond, but could do so earlier.

For retronasal odorant presentations, an odorant presentation container was placed bottom side-down on the subject's extruded tongue after the subject put on Flents<sup>®</sup> nose plugs to prevent orthonasal identification. Subjects were then told to bring the odorant presentation container into the mouth by retracting the tongue. They were then asked to bite gently on the edges of the odorant presentation container (the wall of the outer cylinder),

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

close their mouth, remove the nose plugs, breathe normally, and minimize any tongue or mouth movements. This sequence, which was developed after consultation with a general dentist, was designed to position odorants beneath the posterior nares of the nasopharynx by locating the odorant presentation container in the vicinity of a subject's rear molars, to avoid taste stimulation by separating the odorant from the tongue, and to minimize direct trigeminal stimulation by preventing contact between the odorant and the gum or cheek.

Retronasal stimuli were presented this way for 10 s (Experiment One) or 15 s (Experiments Two through Four) during both retronasal identification training and during retronasal odorant identification evaluation and testing sequences (see below). A subject had the full 10 or 15 s to respond during evaluation or testing trials, but could terminate presentation earlier by putting on the nose plug and sticking out their tongue if they were ready to indicate the identity of the odorant.

Main Experiment Designs

Each of the experiments included four successive steps: i) odorant identification training employing either a retronasal or an orthonasal stimulus delivery route; ii) evaluation of odorant identification learning to criterion, involving the same stimulus delivery route (retronasal or orthonasal) used in training; iii) For those subjects who had achieved the odorant identification criterion (see below), odorant identification testing involving the stimulus delivery route that was not used during training and criterion evaluation. That is, if training and criterion evaluation had utilized orthonasal presentations, then the odorant identification testing of step iii would utilize retronasal presentations. No corrections were provided. Only the first identification was accepted. Those subjects who did not achieve the odorant identification criterion of step ii were thanked for their participation, were not tested in step iii, and did not continue further in the experiment; iv) For those subjects who had achieved the odorant identification criterion and had had their odorant identification accuracy tested in step iii, odorant identification testing was now done involving the stimulus

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

delivery route than was used during training and criterion testing. No corrections were provided. Only the first identification was accepted.

In Experiments One and Two, the above four steps occurred twice, first with orthonasal training (first half of experiment) and later with retronasal training (second half of experiment). This was done to both reveal possible differences in correct odorant identifications with odorants presented by retronasal or orthonasal routes and to allow a comparison of the effectiveness of orthonasal versus retronasal training for odorant identification. The designs of Experiments One and Two differed mainly in that instruction in retronasal breathing was introduced prior to any odorant identification training in Experiment Two. There were no subjects in common between Experiments One and Two.

Experiment Three used a single sequence of the above four training, learning evaluation, and testing steps, preceded by instruction in retronasal breathing, in order to verify the effect of retronasal breathing instruction observed in Experiment Two. Three of the 7 subjects in Experiment Three had also participated in Experiment Two.

In Experiment Four, the above four steps were followed by instruction in retronasal breathing, then practice with the breathing technique, and finally a second series of retronasal and orthonasal testing. Experiment Four was designed to compare retronasal and orthonasal odorant identifications before and after instruction and practice in retronasal breathing. None of the subjects in Experiment Four had participated in any of the three previous experiments.

All components of an experiment, including the screening, were completed during a single session for each subject. A complete session of Experiments One or Two required 45 to 90 minutes, depending upon the number of odorant presentations during training and during evaluation of odorant identification learning to criterion. Experiments Three or Four sessions required between 25 and 50 minutes, as a function of the same factors.

Odorant Identification Training

**Orthonasal and retronasal odorant identification**  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

During the odorant identification training step, four odorant stimuli were presented in a fixed order as in Rozin (1982); subjects proceeded through a sequence of stimulus presentations, with each odorant given on 3 of every 12 presentations (1, 2, 3, 4; 1, 2, 3, 4; 1, 2, 3, 4; 1, 2, 3, 4). Subjects were told the numerical identifier of the odorant (number 1, 2, 3, or 4) before, during, and after each identification training presentation. Subjects attempted to learn to assign the identification numbers 1, 2, 3, and 4 to the four odorants. Numerical identifiers rather than names were used for odorants, following Rozin's (1982) method, since this approach would permit within-subjects comparisons between identifications based upon retronasal and orthonasal pathways, and should both avoid the odorant name recall difficulties that have been reported (Cain and Rabin, 1984) and present a minimal cognitive task to the subjects. For Experiment One, a minimum of 12 and a maximum of 24 odorant identification training presentations were done, with subjects permitted to end training after the initial 12 presentations. In Experiments Two, Three, and Four, subjects could stop during the identification training presentations whenever they felt they knew the odorants' numerical identities. This flexibility in odorant identification training was introduced because, in Experiment One, some subjects had complained about being required to receive at least 3 presentations of each odorant during this training.

Evaluation of Odorant Identification Learning to Criterion

In order to ascertain which subjects had thoroughly learned the numerical identifications of the odorant, after completing odorant identification training, all subjects went through 2 or more identification learning evaluation sequences of 12 random order presentations of the odorants, with each of the four odorants presented three times, and were asked to identify each odorant by number after its presentation. The same odorant presentation route that had been used for identification training was used for criterion evaluation. If subjects made a mistake, they were corrected verbally (that was number 1, not 2). Subjects were required to complete two consecutive sequences of 12 presentations with

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

at most one identification mistake in each sequence in order to proceed to the main testing phases, steps iii and iv. Subjects who did not reach the criterion within the maximum number of random odorant sequences were thanked for their participation and excused from the experiment. In Experiment One, a maximum of 8 criterion sequences was given. For Experiments Two and Three, the initial three subjects also had a maximum of 8 possible identification criterion sequences, but the maximum number of sequences for identification criterion evaluation was decreased from 8 to 6 for subsequent subjects. The reduction in the maximum number of identification criterion evaluation sequences was made after it was observed that no subjects who required more than 6 sequences had reached criterion. In Experiment Four, all subjects had a maximum of 6 identification criterion evaluation sequences available.

Odorant Identification Testing

Subjects who satisfied the criterion for odorant identification learning were next tested to determine their ability to identify the odorants. The 4 stimuli were presented in random order 3 times each for 12 presentations, and subjects were asked to identify them by numbers 1 to 4. Only the first identification was accepted. No corrections were provided. Odorant identification was tested using both retronasal and orthonasal presentation routes in the main testing phase of every experiment. The results of the odorant identification testing are the primary data of the experiments.

Statistics

Non-parametric statistics were appropriate given the sample sizes and nature of all four experiments. Outcomes of experiments were characterized using medians and semi-interquartile ranges, while the presence of statistically significant differences associated with orthonasal versus retronasal testing was evaluated using the Wilcoxon Signed Rank Test. Whenever multiple comparisons were made, Wilcoxon Signed Rank Test p-values were

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

corrected using Bonferroni layering (Darlington, 1990). For those experiments in which retronasal and orthonasal testing were done under more than one condition, overall consistency was evaluated with the Friedman Two-way Analysis of Variance by Ranks.

## EXPERIMENT ONE

Subjects: There were ten men and five women.

1st Half of Experiment: Orthonasal identification training and criterion evaluation were followed by retronasal and then orthonasal identification testing.

### 2nd Half of Experiment

The second half of the experiment was basically the reverse of the first. Those subjects who had satisfied the orthonasal identification learning criterion of the first half of Experiment One and had then been tested for retronasal and orthonasal identification, next proceeded to the retronasal identification training that began the second half of Experiment One. For those subjects who met the subsequent retronasal identification criterion evaluation, odorant identification testing was done with the stimuli first presented orthonasally and then retronasally.

## RESULTS

13 of the 15 subjects (5 women; 8 men) satisfied the orthonasal identification learning criterion (Table I), and proceeded to the testing steps of the first half of the experiment. In the retronasal identification testing, the median number of correct identifications was 9, with a semi-interquartile range (SIR) of 1.38. For the orthonasal testing that followed, median correct identifications = 12, SIR = 0.38 (Table I). The

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

difference was statistically significant, Wilcoxon Signed Rank Test,  $p = 0.0034$ , Bonferroni-corrected.

The 13 subjects whose testing results are presented above then proceeded to the retronasal identification training that began the second half of Experiment One. Nine of these 13 subjects (2 women; 7 men) satisfied the retronasal identification learning criterion (Table I). For the subsequent orthonasal testing, these 9 subjects had a median of 12 correct identifications,  $SIR = 0.0$ . For the retronasal testing that followed, median correct identifications = 11,  $SIR = 1.0$  (Table I). This difference was statistically significant, Wilcoxon Signed Rank Test,  $p = 0.038$ , Bonferroni-corrected.

After orthonasal training and criterion evaluation, the number of correct identifications made during retronasal testing was, for every subject, less than the number of correct identifications made during orthonasal testing (Table I). For the testing that followed the retronasal training and criterion evaluation in the 2nd half of Experiment One, the number of correct identifications made during orthonasal testing was, for every subject, greater than or equal to the number of correct identification made during retronasal testing. Equal numbers correct, 12 in each case, occurred during the testing steps of the second half of Experiment One for 4 of these 9 subjects (Table I). The numbers of correct identifications were consistent across the 9 subjects who completed both halves of the experiment for the 2 orthonasal and 2 retronasal tests,  $p = 0.001$ , Friedman Two-way Analysis of Variance by Ranks,  $F_T = 15.433$ ,  $df = 3$ .

## DISCUSSION

Experiment One demonstrated that, as Rozin (1982) had suggested, differences in the identifiability of odorants do occur depending upon whether stimuli arrive through an orthonasal or a retronasal route. A consistent superiority of identifications during orthonasal testing, independent of the identification training being orthonasal or retronasal, was also observed. The latter asymmetrical outcome is incompatible with two aspects of

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

Rozin's (1982) 'olfactory duality' model for perception of retronasal and orthonasal odorants. One incompatibility is the substantial transfer between orthonasal identification training and retronasal testing. Little or no transfer would be expected if orthonasal and retronasal inputs represented the stimulus delivery pathways of two quite separate olfactory systems. A second incompatibility is the asymmetry between incidence of correct responses to orthonasal versus retronasal stimulation. If retronasal and orthonasal inputs were equipotential, then whatever the degree of independence of retronasal and orthonasal systems, correct retronasal identifications following orthonasal training should be comparable in number to those for correct orthonasal identifications following retronasal training.

Experiment One had been designed to assess the existence and nature of differences between odorant identifications based upon retronasal and orthonasal inputs during normal, non-sniffing inhalation and exhalation, but did not seek to identify or test possible mechanisms for any such differences. After the retronasal and orthonasal differences were observed, Experiment Two was planned as an attempt to decrease any possible insufficiency in retronasal odorant input, and thus to probe differences in odorant access under normal breathing conditions as a potential reason for the orthonasal-retronasal asymmetry observed in Experiment One. This was done by introducing a modified retronasal breathing technique in Experiment Two, prior to the main experiment.

The possibility that the observed differences in odorant identification could be due to the order of stimulus presentations received by the subjects during testing was controlled for in Experiment One by random stimulus orders both between and within subjects during all testing. However, it could be that some random orders disadvantaged certain subjects. This possibility was addressed in Experiment Two by using one randomly determined sequence of stimulus orders for testing all subjects during the first half of an experiment, and another random sequence of stimulus orders for odorant identification testing during

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

the second half. This procedure still presented a random series of stimuli within subject, but now the same random series were presented to all subjects.

## EXPERIMENT TWO

Subjects. There were two men and five women. None had served in Experiment One.

### Retronasal Practice

First, before odorant identification training began, in order to familiarize subjects with the method of retronasal odorant presentation, subjects practiced retronasal presentations with an empty odorant presentation container, after a demonstration by an experimenter. Subjects were told by an experimenter, "I'm putting on the nose plug so I can't smell anything. Now the odorant presentation container is placed on the end of my tongue like so, after which I'll pull it back and bite down on the edges of it with my molars, take off the nose plug and breathe through my nose." An experimenter performed this on himself. He held the odorant presentation container inside his mouth for several seconds, then put the nose plug on, then opened his mouth and removed the container. This demonstration was done twice by an experimenter, and then done by the subject up to 5 times. These demonstration and practice procedures were instituted because of minor difficulties earlier subjects had had in holding and manipulating odorant presentation containers during retronasal breathing.

### Retronasal Breathing Instruction

Second, in an attempt to increase the efficiency of retronasal smelling, subjects were instructed in how to breathe during a retronasal presentation. Subjects were told by an experimenter: "I'm going to teach you how to breathe in the retronasal part of the experiment. First, I tense my stomach as I exhale, kind of forcing the air out of my lungs with my mouth open, but still controlling the rate of air flow so as to be normal and not all at

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

once. Also, I don't take a deep breath before I exhale; I breathe out where I normally would, after an ordinary inhale. Watch me [an experimenter did this with mouth open, making appropriate wheezing sound]. Did you hear that sound when I exhale? [an experimenter now repeated the demonstration]." Next an experimenter asked the subject to do what he had just done, several times if necessary, until the subject did it correctly. Then an experimenters said, "Now I'm simply going to close my mouth when I exhale and let the air funnel itself out my nose with the same breathing technique. I'm not going to outwardly sniff; again, the exhale comes from the diaphragm and my stomach muscles are tensed." An experimenter did this twice, then told the subject to do the same. An experimenter allowed the subject several tries until he/she did it 'correctly'. 'Correctness' was inferred from watching the practicing subject while listening for a wheezing which indicated successful modification of retronasal breathing.

Main experiment

Experiment Two general procedures were as in Experiment One, except the same set of randomized sequences of odorant presentations was used for the identification criterion evaluation and the main retronasal and orthonasal testing across all subjects for the first half of Experiment Two; another set of randomized sequences, for the main orthonasal and retronasal testing of the second half of Experiment Two.

RESULTS

After orthonasal identification training, all 7 subjects satisfied the identification learning criterion (Table II). In the subsequent retronasal testing, median number correct identifications = 10, SIR = 1.88. For the orthonasal testing that followed, median correct identifications = 12, SIR = 0.125. This difference was statistically significant, Wilcoxon Signed Rank Test,  $p = 0.042$ , Bonferroni-corrected.

**Orthonasal and retronasal odorant identification**  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

The 7 subjects whose testing results are presented above then proceeded to the retronasal identification training that began the second half of Experiment Two. After the retronasal identification training, 6 of the 7 subjects (4 women; 2 men) satisfied the criterion with retronasal presentations (Table II). For the subsequent orthonasal testing, median correct identifications = 12, SIR = 0.5. For the retronasal testing that followed, median correct identifications = 12, SIR = 0.75. These outcomes were not significantly different, Wilcoxon Signed Rank Test,  $p = 0.655$ , Bonferroni-corrected.

After orthonasal training and criterion evaluation, the number of correct identifications made during orthonasal testing was, for every subject, greater than or equal to the number of correct identifications made during retronasal testing (Table II). Equal numbers correct, 12 in each case, occurred during testing for 2 of these 7 subjects. For the testing that followed the retronasal training and criterion evaluation in the 2nd half of Experiment Two, the number of correct identifications made during orthonasal testing was greater than the number of correct identifications made during retronasal testing for 1 subject, less than the number of correct identifications made during retronasal testing for 1 subject, and equal to the number of correct identification made during retronasal testing, 12 in each case, for 4 of these 6 subjects. The numbers of correct identifications were not consistent across the 6 subjects who completed both halves of the experiment for the 2 orthonasal and 2 retronasal tests,  $p = 0.308$ , Friedman Two-way Analysis of Variance by Ranks,  $F_r = 3.6$ ,  $df = 3$ .

## DISCUSSION

After the orthonasal training of Experiment Two, which had produced sufficient learning for all subjects to meet the criterion, a significant difference between the number of correct identifications made during retronasal and orthonasal testing persisted, despite the introduction of a breathing modification intended to decrease or eliminate a possible insufficiency in retronasal odorant input. The difference in the number of correct responses

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

after orthonasal training confirms the orthonasal testing superiority observed in Experiment One, and could indicate that the modified retronasal breathing that was introduced in Experiment Two, and its underlying rationale, were inappropriate. However, after the retronasal training that began the second half of Experiment Two, no statistically significant difference in correct identifications remained, while the median numbers of correct identifications were now at the upper limit for both orthonasal and retronasal testing. The possibility of ‘ceiling effects’ is addressed in the General Discussion.

Subjects had had prior experience when tested during the 2nd, retronasal-training half of Experiment Two, since they had already participated in the testing of the first half of the experiment. If no additional data were available, it might be reasonable to consider the prior experience responsible for at least a portion of the disappearance of orthonasal superiority during the 2nd half of Experiment Two. However, the subjects of Experiment One were as experienced as those of Experiment Two when tested during the 2nd, retronasal-training half of Experiment One. Nonetheless, the Experiment One subjects, who used normal retronasal exhalation, continued to demonstrate a significant orthonasal superiority during the 2nd half of Experiment One, while the subjects of Experiment Two, having received instruction in retronasal breathing, no longer exhibited a significant orthonasal superiority during the 2nd, retronasal-training half.

There appears to be an interaction between odorant delivery pathway during identification training, breathing method, and number of correct identifications during testing. The modified retronasal breathing technique introduced in Experiment Two seems sufficient to produce equivalent retronasal and orthonasal testing performances under the conditions of the present experiments if identification training utilizes a retronasal pathway, but fails to yield commensurate numbers of correct identifications if orthonasal access is used for identification training.

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

A replication of the retronasal training and modified breathing combination would be desirable in order to confirm that no significant disparity occurs between orthonasal and retronasal correct identifications under these conditions. Experiment Three was designed to provide this replication.

### EXPERIMENT THREE

Subjects. There were four men and six women.. Three of the subjects had also participated in Experiment One, which was executed approximately seven months before Experiment Three.

#### Main experiment

The procedure used was the same as that in the second half of the Experiment Two, preceded by the retronasal odorant presentation container practice and breathing instruction as in Experiment Two. That is, for Experiment Three, subjects first received practice in using the odorant presentation container inside the mouth, and were also instructed in retronasal breathing, both as in Experiment Two, and then received retronasal identification training and retronasal identification criterion evaluation followed by orthonasal and then retronasal testing.

### RESULTS

After the retronasal identification training, 8 of the 10 subjects (6 women, 2 men) satisfied the identification learning criterion (Table III). In the subsequent orthonasal testing of these 8 subjects, median correct identifications = 12, SIR = 0. For the retronasal testing that followed, median correct identifications = 11.5, SIR = 1.0. This difference was not statistically significant, Wilcoxon Signed Rank Test,  $p = 0.066$ . For all subjects, 12 correct identifications were made on their orthonasal testing sequence (Table III). The number of correct identifications made during orthonasal testing was greater than the

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

number of correct identification made during retronasal testing for 4 subjects, and equal to the number of correct identification made during retronasal testing, all correct under both conditions, for the other 4 subjects (Table III).

## DISCUSSION

In Experiment Three, in which instruction in retronasal breathing was used in conjunction with retronasal identification training, there was no significant difference in the number of correct odorant identifications during orthonasal and retronasal testing. It appears that the retronasal breathing instruction makes possible an important change in the effectiveness of retronasal odorant stimulus delivery, perhaps by improving the efficiency of odorant access to the olfactory mucosa.

However, the existence of a change in effectiveness of retronasal stimulation following the retronasal breathing instruction, although a logical inference, has not been directly demonstrated since subjects in Experiments Two and Three were instructed in the retronasal breathing technique before any identification training began, while those of Experiment One never used modified retronasal breathing. In addition, the extent to which orthonasal identification training precludes any action of altered retronasal breathing is unclear. Therefore, Experiment Four was designed to both directly examine the degree to which introduction of retronasal breathing instruction increased the number of correct retronasal identifications in comparison with those made prior to such instruction, and to assay whether retronasal breathing could be made sufficiently effective under conditions of orthonasal training to eliminate the orthonasal testing superiority observed in Experiments One and Two.

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

EXPERIMENT FOUR.

Subjects. Eight men and four women. None had participated in Experiments One, Two, or Three.

Main experiment

Subjects received orthonasal odorant identification training, orthonasal odorant identification learning to criterion evaluation, and two testing steps, namely retronasal testing and orthonasal testing, as in the first half of Experiment Two, with the following three changes. 1) No retronasal breathing instruction was done at the beginning of the main experiment. 2) After orthonasal odorant identification training and the orthonasal odorant identification criterion evaluation, there were two pairs of retronasal and orthonasal testing steps. The subjects received retronasal breathing instruction and then practice in using it (see #3, below) after the first pair of retronasal and orthonasal tests and before the second pair. 3) After retronasal breathing instruction identical to that of Experiments Two and Three, subjects were then told “Okay, now let’s try it. This is chocolate.” An experimenter showed subjects an actual odorant presentation container with minced Hershey's Unsweetened Baking Chocolate in it (subjects were not asked to close their eyes). Chocolate was used for this supplement to the retronasal breathing instruction because it would not be a stimulus in any of the Experiment Four training, evaluation, or testing steps. An experimenter then said “Now smell this sample as you’ve done in the retronasal breathing instruction part of the experiment.” Subjects were allowed between 10 and 15 s for the presentation of this known retronasal stimulus, and were asked during that interval “Can you smell it better that way?” The answer was invariably ‘yes’, as communicated through a nod of the head or some other nonverbal expression. Subjects were able to provide this qualitative comparison of their retronasal olfaction after, versus before, instruction and practice in retronasal breathing because the retronasal breathing

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

instruction and practice of Experiment Four followed the first pair of retronasal and orthonasal odorant identification tests.

RESULTS

After the orthonasal identification training, all 12 subjects satisfied the orthonasal identification learning criterion (Table IV) and participated in the main experiment. In the subsequent initial retronasal testing, median correct identifications = 10, SIR = 1.5. For the initial orthonasal testing that followed, median correct responses = 12, SIR = 0.5. This difference was statistically significant, Wilcoxon Signed Rank Test,  $p = 0.018$ , Bonferroni-corrected. The number of correct identifications made during initial retronasal testing was less than the number of correct identification made during initial orthonasal testing for 11 of the subjects (Table IV).

After the retronasal breathing instruction and practice of Experiment Four that followed the initial testing, the second retronasal identification test gave a median number of correct responses of 11.5, SIR = 1.5. For the second orthonasal identification testing that came next, median number of correct identifications equaled 12, SIR = 0.5. This difference was not statistically significant, Wilcoxon Signed Rank Test,  $p = 0.149$ , Bonferroni-corrected. Five subjects gave more correct identifications during their 2nd orthonasal test than during their 2nd retronasal test, 3 subjects gave equal numbers of correct identifications during their 2nd orthonasal and retronasal tests, both numbers correct being 12, and 4 subject gave fewer correct odorant identifications during their 2nd orthonasal test than during their 2nd retronasal test (Table IV).

For the majority of the subjects the number of correct retronasal odorant identifications made during testing after the retronasal breathing instruction and practice was greater than the number of correct identifications made during the initial retronasal testing, which occurred before retronasal breathing instruction (Table IV), but this difference did not reach statistical significance,  $p = 0.058$ , Bonferroni-corrected.

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

The numbers of correct identifications were consistent across the 12 subjects for both halves of Experiment Four for the 2 retronasal and orthonasal tests,  $p = 0.014$ , Friedman Two-way Analysis of Variance by Ranks,  $F_T = 10.625$ ,  $df = 3$ .

DISCUSSION

The initial portion of Experiment Four essentially replicated the first half of Experiment One in both general procedures and in results, although the two experiments had no subjects in common and were separated in time by more than 12 months. As had been the case in Experiment One, with natural retronasal breathing and orthonasal identification training and learning to criterion evaluation, the number of correct odorant identifications in Experiment Four was substantially and significantly greater under orthonasal testing than under retronasal testing. This orthonasal testing superiority was reflected in the individual patterns of 11 of 12 Experiment Four subjects. Since all subjects in Experiment Four had attained the orthonasal criterion, the higher orthonasal performance during initial odorant identification testing cannot be attributed to selection of subjects with special orthonasal prowess.

A substantial improvement in the accuracy of retronasal identifications followed the retronasal breathing instruction and practice which were introduced after the initial pair of retronasal and orthonasal identification tests of Experiment Four. Results with retronasal testing no longer differed significantly from those with orthonasal testing. At the individual level, increases in the number of correct identifications under retronasal testing occurred in 59% of the subjects after the retronasal breathing instruction.

Perceptual learning due to the first pair of retronasal and orthonasal tests of Experiment Four could have contributed to the greatly increased retronasal accuracy during the second pair of tests. However, the subjects of Experiment One not only also had an opportunity for such perceptual learning during the first half of that experiment but also were trained on retronasal identification during the second half of the experiment.

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

Nonetheless, in the absence of instruction in retronasal breathing, correct retronasal identification scores during Experiment One remained significantly below orthonasal scores.

It appears that the retronasal breathing instruction and practice of Experiment Four, in which subjects had an opportunity to practice their just-acquired modified retronasal breathing using an identified odorant, was more effective than the instruction without practice that had been used in Experiment Two. After the practiced retronasal breathing technique of Experiment Four, an orthonasal superiority was no longer evident. The implication is that orthonasal and retronasal odorant access pathways may differ only in some aspect of ease or efficiency of odorant access or delivery.

### **GENERAL DISCUSSION**

Comparisons of orthonasal and retronasal responses to gaseous odorants, per se, require that only vapor phase stimuli be delivered. This was accomplished in the present experiments by using odorant sources in solid form which were placed in the vicinity of the anterior or posterior nares using unique odorant delivery containers. The design of these containers permitted unimpeded solid-phase to vapor phase transition of the odorants but prevented direct contact between the solid-phase odorants and any underlying or surrounding tissues. For the orthonasal presentations, these odorant presentation containers provided little special advantage other than ease of situating odorants immediately below the nostrils. However, containers of the very same design could be positioned in the oral cavity such that the odorants were located beneath the posterior nares of the nasopharynx, but without any direct contact between the odorants and the tongue or soft tissue of the mouth. Consequently, the retronasal stimulation method that was employed excluded the possibility

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

of the lingual taste or trigeminal effects that have been necessary components of those investigations that introduced liquids directly into the mouth.

The data reported in these experiments do not verify proposals that orthonasal and retronasal olfaction are completely separate and functionally quite different olfactory systems. No support was found for the "olfactory duality" hypothesized by Rozin (1982), with "olfactory duality" understood to require that learning to identify by a retronasal route a set of odorants would provide little if any benefit when orthonasal identification was tested. Rozin had argued that there exists a qualitative difference in the perception of an odorant depending on whether it is perceived orthonasally or retronasally. If a qualitative difference in olfactory perception is taken to specify only that identical suprathreshold odorants will necessarily elicit different responses solely as a function of stimulus presentation by an orthonasal or a retronasal route, then the present experiments do not substantiate this prediction.

However, an alternative interpretation of qualitative difference in the perception of odorants would require that the odorants be matched for perceived intensity, so that an observation of unequal accuracy in identification via retronasal and orthonasal routes would necessarily be based upon qualitative differences between the odorants. The odorants used in the present experiments were not explicitly matched for equal perceived intensity. Physical properties of the odorants together with the retronasal vapor phase stimulation technique precluded perceived intensity matching. The odorants were familiar, common substances in their usual solid forms. The retronasal stimulation technique required that the odorants themselves be placed in presentation containers within the mouth, with the mouth closed. Because of the absence of matched perceived intensities, it is possible that subjects could have learned the numerical identifiers assigned to each odorant based upon differences in perceived intensity rather than qualitative differences between the odorants.

**Orthonasal and retronasal odorant identification**  
**Pierce and Halpern *Chemical Senses*, 21, 529-543, 1996.**

Future experiments could attempt to resolve this question by using liquid odorants diluted such that a perceived intensity match was produced.

Mozell (1971) had previously proposed that the direction of odorant flow across the olfactory mucosa, from posterior nares toward anterior nares, or the reverse, could be an important factor in the discrimination of odorants. This has been referred to as the gas chromatographic model of olfaction (Engen, 1982), and is thought to describe a physical reality at the olfactory receptor epithelium irrespective of the extent to which it is involved in olfactory coding (Hornung, Mozell, and Serio, 1980; Hornung and Mozell, 1985). Mozell (1971) noted " ... as a result of their differential attraction to the media of the olfactory mucosa, the molecules of some chemicals progress more rapidly and in greater numbers along the mucosal sheet than do the molecules of other chemicals. The receptors could then simply signal these molecular movements ...." In effect, the Mozell (1971) approach, which posited an arrangement of functionally separate olfactory systems which shared the same sensory epithelium, could also be considered as a form of dual olfactory systems.

However, Mozell (1971) neither asserted that the receptor cells of the olfactory mucosa had identical properties nor did he dismiss the sensory coding importance of the selective sensitivity of individual olfactory receptor neurons: "... evidence is now at hand to support the possibility of two mucosal mechanisms upon which olfactory discrimination may be based: (1) a loose sensitivity of the receptors themselves; (2) a spatio-temporal encoding based upon the relative distribution and speed of travel of the molecules across the mucosa." In agreement with Mozell (1971), many (but not all, e.g., Chanel, 1987) current workers advocate hybrid olfactory coding models which combine the "inherent" selective sensitivity of loosely grouped olfactory receptor neurons and the "imposed patterning" due to the direction of odorant access and flow and sorptive interactions between odorants and the mucosa (e.g., Kauer, 1980; Kubie, Mackay-Sim, and Moulton, 1980; Getchell, Margolis,

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

and Getchell, 1984; Hornung and Mozell, 1985; Kauer, 1987; Cain, 1988; Holley, 1991; Kauer, 1991).

The relationship between the data of the present experiments and the Mozell (1971) model is unclear. Comparable identification accuracy for retronasal and orthonasal routes was achieved when retronasal breathing instruction and practice were introduced. This outcome might not be expected from the gas chromatographic model of olfaction, but, as already noted, the lack of matched perceived intensity for the odorants does not allow a interpretation that identifications were based only upon qualitative differences.

The odorants of the present experiments were selected to be readily discriminable from each other, without sniffing, during orthonasal presentations. This may account for the 100% accuracy of orthonasal identification by the majority of subjects who had met the orthonasal criterion after orthonasal training, with no subject scoring less than 80% correct on orthonasal testing. Although the achievement of criterion-level odorant identification learning was more difficult when retronasal criterion evaluation was done after retronasal training, no subject who had met the retronasal criterion scored less than 60% correct upon retronasal testing; the majority produced correct identifications on 80% or more of the retronasal testing trials. When retronasal breathing instruction was provided, no subject was less than 75% correct upon retronasal testing, with the majority providing correct identifications on 92% or more of their retronasal testing trials.

These very high levels of performance probably represent ‘ceiling effects’, and if so must limit the conclusions that may be drawn from the data of the present experiments. It is possible, and indeed likely, that the use of odorants having greater similarity than those of the present experiments would reveal results different than those obtained in these experiments. For example, if normal retronasal breathing were employed together with rather similar stimuli, the orthonasal superiority between training with input through one pair of nares and testing with input through the other nares would likely be larger than was

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

observed in the present experiments, while learning to identify to a criterion odorants with retronasal input would be even more difficult than the present data indicate. If orthonasal sniffing were permitted, retronasal performance would probably be even more inferior. In addition, instruction in and practice with a modified retronasal breathing technique, which was able to eliminate the retronasal versus orthonasal disparity for the stimulus arrays of the present experiments, might be unable to do so for more similar stimuli.

Given the proposed importance of retronasal olfaction in the oral perception of food and drink (e.g., Gibson, 1966; Roberts and Acree, 1995), retronasal odorant identification accuracy that is inferior to orthonasal accuracy under normal breathing conditions may seem counterintuitive. Nonetheless, this was repeatedly observed in the present study. Inferior retronasal sensitivity had been measured in a prior report of higher retronasal than orthonasal vapor phase odorant thresholds (Voirol and Daget, 1986). Several factors may serve to explain or rationalize these inequalities between responses to odorants presented by orthonasal or retronasal routes. One such factor could be the absence of tongue movements in the studies that have used retronasal vapor phase stimulation. Tongue movements and swallowing are correlated with greater chemosensory perception of intraoral liquids (Burdach and Doty, 1987). Prevention of such intraoral movements in the above studies may have produced diminished retronasal acuity and sensitivity. The present authors' personal experiences indicate that swallowing with the mouth closed produces a brief and pronounced movement of air out of the nostrils. Under normal circumstances, this expiratory event, which has some resemblance to the modified retronasal breathing taught in the present study, may constitute a "retronasal sniff."

A second factor in the observed inferiority of retronasal olfaction may be the stimulus concentrations that were selected for study. Normally, retronasal olfaction of food and liquids may encounter more concentrated odorants than does orthonasal olfaction, and therefore it may function best over a higher concentration range. If so, examining retronasal

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

and orthonasal olfaction using concentrations selected for orthonasal olfaction may be excessively limiting. It is desirable to explore retronasal olfaction as a primary subject of study, rather than only for comparison to orthonasal olfaction.

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Orthonasal and retronasal odorant identification  
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Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

**TABLES**

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

**Table I.** Experiment one subjects' gender and number of correct retronasal and orthonasal identifications after orthonasal training and criterion testing (1st) and after retronasal training and criterion testing (2nd)

| GENDE<br>R | Number of Correct Identifications |           |                           |           |
|------------|-----------------------------------|-----------|---------------------------|-----------|
|            | After Orthonasal Training         |           | After Retronasal Training |           |
|            | 1st Retro                         | 1st Ortho | 2nd Ortho                 | 2nd Retro |
| female     | 8                                 | 12        | MRC                       | MRC       |
| female     | 11                                | 12        | MRC                       | MRC       |
| male       | 10                                | 11        | 12                        | 11        |
| male       | 11                                | 12        | 12                        | 12        |
| female     | 10                                | 12        | 11                        | 10        |
| male       | 8                                 | 12        | 12                        | 12        |
| male       | 7                                 | 11        | 12                        | 12        |
| female     | 7                                 | 10        | MRC                       | MRC       |
| male       | 9                                 | 12        | MRC                       | MRC       |
| male       | 8                                 | 12        | 12                        | 12        |
| male       | 9                                 | 12        | 12                        | 10        |
| female     | 11                                | 12        | 12                        | 10        |
| male       | 4                                 | 12        | 12                        | 10        |

1st Retro = 1st Retronasal Identification Test; 1st Ortho = 1st Orthonasal Identification Test

2nd Ortho = 2nd Orthonasal Identification Test; 2nd Retro = 2nd Retronasal Identification

Test; MRC = Missed Retronasal Criterion

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

**Table II.** Experiment two subjects' gender and number of correct retronasal and orthonasal identifications after retronasal breathing instruction, followed by orthonasal identification training and criterion testing (1st) and then retronasal training and criterion testing (2nd).

| GENDER | Number of Correct Identifications |           |                           |           |
|--------|-----------------------------------|-----------|---------------------------|-----------|
|        | After Orthonasal Training         |           | After Retronasal Training |           |
|        | 1st Retro                         | 1st Ortho | 2nd Ortho                 | 2nd Retro |
| male   | 10                                | 12        | 12                        | 12        |
| female | 12                                | 12        | 12                        | 12        |
| female | 8                                 | 12        | MRC                       | MRC       |
| female | 12                                | 12        | 11                        | 12        |
| female | 6                                 | 12        | 12                        | 12        |
| female | 9                                 | 11        | 11                        | 9         |
| male   | 11                                | 12        | 12                        | 12        |

1st Retro = 1st Retronasal Identification Test

1st Ortho = 1st Orthonasal Identification Test

2nd Ortho = 2nd Orthonasal Identification Test

2nd Retro = 2nd Retronasal Identification Test

MRC = Missed Retronasal Criterion

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

**Table III.** Experiment three subjects' gender and number of correct orthonasal and retronasal identifications after retronasal breathing instruction, identification training, and criterion testing.

| GENDER  | Number of Correct |            |
|---------|-------------------|------------|
|         | Identifications   |            |
|         | Orthonasal        | Retronasal |
| male*   | 12                | 11         |
| male*   | 12                | 10         |
| female  | 12                | 12         |
| female  | 12                | 12         |
| female  | 12                | 12         |
| female* | 12                | 9          |
| female  | 12                | 12         |
| female  | 12                | 11         |

Orthonasal = Orthonasal identification test

Retronasal = Retronasal identification test

\* Had participated in Experiment One.

Orthonasal and retronasal odorant identification  
Pierce and Halpern *Chemical Senses*, **21**, 529-543, 1996.

**Table IV.** Experiment four subjects' gender and number of correct retronasal and orthonasal identifications after orthonasal training and criterion testing before (1st) and after (2nd) retronasal breathing instruction and practice.

| GENDER | Number of Correct Identifications |           |           |           |
|--------|-----------------------------------|-----------|-----------|-----------|
|        | 1st Retro                         | 1st Ortho | 2nd Retro | 2nd Ortho |
| female | 10                                | 12        | 9         | 12        |
| male   | 11                                | 12        | 11        | 10        |
| male   | 10                                | 12        | 12        | 11        |
| male   | 11                                | 12        | 12        | 12        |
| male   | 8                                 | 12        | 12        | 12        |
| male   | 11                                | 9         | 12        | 11        |
| male   | 9                                 | 12        | 8         | 12        |
| female | 11                                | 12        | 12        | 12        |
| female | 11                                | 12        | 12        | 11        |
| male   | 8                                 | 9         | 8         | 9         |
| female | 6                                 | 11        | 10        | 11        |
| male   | 9                                 | 11        | 9         | 12        |

1st Retro = 1st Retronasal Identification Test

1st Ortho = 1st Orthonasal Identification Test

2nd Retro = 2nd Retronasal Identification Test

2nd Ortho = 2nd Orthonasal Identification Test