Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

Maggie Phan	Date

The effects of chemical properties and nasal air flow patterns on retronasal responses to odorants in the rat.

By

Maggie Phan
Master of Science

Graduate Division of Biological and Biomedical Sciences
Nutrition and Health Sciences

_____

John W. Scott, Ph.D.
Advisor

_____

Ping Chen, Ph.D.
Committee Member

_____

Hubert W. Vesper, Ph.D.
Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____

Date

The effects of chemical properties and nasal air flow patterns on retronasal responses to odorants in the rat.

By

Maggie Phan
B.A. University of Pennsylvania, 2004

Advisor: John W. Scott, Ph.D.

An abstract of
A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of Master of Science in Nutrition and Health Sciences
2010

Abstract

The effects of chemical properties and nasal air flow patterns on retronasal responses to odorants in the rat.

By Maggie Phan

In this study of orthonasal and retronasal olfaction, electroolfactograms (EOGs) were measured from the dorsal-medial and lateral regions of the rat olfactory epithelium. Orthonasal olfaction is the process by which odorants enter the nasal cavity from the anterior nares, as in sniffing. Retronasal olfaction is the process of smelling from the mouth, which occurs when odorants from food inside the mouth travel behind the palate to the posterior nares and enter the nasal cavity. Sixteen odorants with a range of solubility were tested, and the effects of single and multiple pulses of odor stimulation were studied. The odorants' molecular properties are important factors to the distribution of responses on the olfactory epithelium as well as the magnitude of the orthonasal and retronasal responses. A set of molecular descriptors related to polarity and solubility, including the Hansen solubility parameter, the electrotopological state, and Henry's Law constant, were compared to the peak negative EOG and to the area under the EOG traces. The multiple pulses provide an initial attempt at simulating the effect in breathing, chewing and swallowing. The EOG responses to the odorants were different during orthonasal and retronasal flow. When odorants travel within the nasal cavity in the orthonasal direction, the responses to the polar odorants are the greatest in the dorsal-medial region of the olfactory epithelium while the responses to many nonpolar odorants are the greatest in the lateral region. When odorants travel within the nasal cavity in the retronasal direction, however, the responses to the polar odorants are greatly reduced at all recording sites compared to responses of polar odorants flowing in the orthonasal direction. The single and triple pulse odorant stimulation had similar relationships with the molecular properties of the odorants. This consistent relationship between calculated properties and odorant response supports the hypothesis that odorant sorption is an important contribution to the differences between orthonasal and retronasal olfaction.

The effects of chemical properties and nasal air flow patterns on retronasal responses to odorants in the rat.

By

Maggie Phan
B.A. University of Pennsylvania, 2004

Advisor: John W. Scott, Ph.D.

A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of Master of Science in Nutrition and Health Sciences
2010

Table of Contents

Introduction

Olfaction begins with the delivery of odor molecules to the nasal cavity. Inside the rat nose, the odor molecules move through a long path of nonolfactory mucosa before arriving at the olfactory epithelium, in which the olfactory receptors are located on the cilia of the olfactory sensory neurons in the mucus. The olfactory sensory neurons project axons to the olfactory bulb, where the axons synapse with the dendrites of the second-order neurons, which project to the olfactory cortex.

During orthonasal olfaction, odorants enter the nasal cavity from the anterior nares, as in sniffing. Retronasal olfaction is the process of smelling from the mouth, which occurs when odorants from food inside the mouth travel behind the palate to the posterior nares and enter the nasal cavity. For both directions of olfaction, the odorants travel along the nasal mucus of the olfactory epithelium and bind to the receptors on the cilia of the olfactory receptor neurons. Human responses to retronasal odor presentation are different from orthonasal presentation. For instance, some cheeses have an unpleasant orthonasal odor, but a pleasant retronasal odor. Patients who have sinonasal disease have better retronasal than orthonasal olfactory function (Landis *et al*., 2003). There are both electrophysiological evidence from human olfactory event-related potentials from the cortical and subcortical regions of the brain

(Heilmann and Hummel, 2004) and magnetic resonance imaging (Small *et al*., 2005) that suggest that the central processing of orthonasal and retronasal information is different.

Retronasal olfaction is a major component of the flavor of food along with taste. The perception of flavor is thus highly influenced by the retronasal olfactory perception. Salivation, chewing, and the temperature change of the food when it enters the mouth all affect the aroma from retronasal olfaction (Linforth *et al*., 2002; Taylor, 1996; Duffy, 2007). Taste is commonly confused for smell. This confusion can occur, for example, when the olfactory cleft at the top of the nasal cavity is blocked from odorants (such as during colds). Only olfaction is diminished, but people may say that they cannot "taste." For humans, food aromas, along with other flavor components are a direct cause of acceptance, rejection, and preferences of foods. They are critical in determining the behavior of human feeding. Altered or lowered retronasal olfactory perception may negatively affect food intake as the enjoyment of food through pleasant flavors is an important influence on food choices and flavor preferences. Diminished retronasal olfactory sensation may decrease flavor sensation, resulting in greater food (particularly sweets, fats, and sugars) intake for satiation. On the other hand, lowered sensation may lead to decreased appetite, which can reduce dietary intake and hence, decreased nutrient intake (de Jong *et*

al., 1999). A problematic factor in the nutritional status of elderly adults is decreased or distorted flavor perception, or loss of retronasal olfactory perception with aging. Anatomical changes as a result of the aging process (de Jong *et al*., 1999; Ship and Weiffenbach, 1993; Weiffenbach *et al*., 1986; Schiffman, 1993; Fukunaga *et al*., 2005;), decline in oral and dental health (Chauncey *et al*., 1984; Burdach and Doty, 1987; Waylar *et al*., 1990; Ship *et al*., 1996; Ship, 1999; Ritchie *et al*., 2002; Bergdahl and Bergdahl, 2002; Ohno *et al*., 2003; du Toit, 2003), illnesses (Ng *et al*., 2004), and usage of medications (Weiffenbach *et al*., 1986; Beidler and Smith, 1991; Berteretche *et al*., 2004) are all things that may contribute to the decline in sensory function.

Rozin (1982) had described olfaction as a dual sensory modality because it senses both objects in the external world (orthonasal) and objects in the mouth (retronasal). He listed three possible mechanistic explanations for the dual modality. (1) Depending on whether the olfactory stimuli are external or within the mouth, olfactory stimuli may be perceived and processed in the brain in two different ways. (2) The olfactory stimuli blend with the oral stimuli, forming a combination of olfactory, gustatory, and tactile input to the brain. (3) Olfactory stimuli that are external and olfactory stimuli that are within the mouth have qualitatively different sensations due to the differential amounts they are sorbed onto the olfactory mucosa. For example, mastication of food inside the mouth

would affect the concentration of olfactory stimuli reaching the olfactory region in the nasal cavity.

I chose to explore the hypothesis that differential sorption of odorants onto the walls of the nasal mucosa related to the direction of air flow, the local air flow rate, and the physicochemical properties of the odorants may contribute to the difference between orthonasal and retronasal olfaction. This differential sorption was observed in several studies of the bullfrog olfactory sac (Mozell, 1964, 1970, 1991; Mozell and Jagodowicz, 1973). Scott *et al*. (2007) tested this hypothesis by recording electroolfactogram (EOG) responses to odorants of various physicochemical properties during orthonasal and retronasal flow. The EOG is a surface-negative electric potential created when odorants bind to the receptors on the cilia of the olfactory sensory neurons in the olfactory epithelium. This study found that nonpolar, hydrophobic odorants are the most effective stimuli during retronasal olfaction.

Odorant sorption is the tendency of odor molecules to adhere to the walls of the nasal mucosa. The sorption of an odorant onto the nasal mucosa can be approximated by water solubility for highly water soluble odorants (Mozell and Hornung, 1985). The solubility can be estimated by the air/water partition, which is a compound's equilibrium between the air and water phases. The air/water partition coefficient is also Henry's Law constant. Sorption is also

likely affected by odorant binding proteins and enzymes, which are secreted by the Bowman's glands located in the olfactory epithelium (Badonnel *et al*., 2009). These contents in the olfactory mucosa increase the solubility of hydrophobic odorants in the mucus (Mozell and Hornung, 1985; Kurtz *et al*., 2004; Ko *et al*., 2010). The water insoluble odorants, though, appear to have greater sorption in the epithelium than expected from their water solubility.

The relationship between odorant sorption and olfactory responses has been predicted by the chromatographic column hypothesis (Mozell, 1970). Mozell recorded summated neural discharges from the olfactory nerve of the bullfrog in response to several odorants representing a range of mucosal sorption strengths. The bullfrog has a tube-like olfactory sac through which the odorants travel. There were two recording sites on the olfactory nerve: the lateral branch innervating the mucosal region near the internal naris and the medial branch innervating the mucosal region near the external naris. The spatial distribution of olfactory responses along the olfactory sac was highly correlated with the odorants' retention times through a polar gas chromatographic column. Odorants that had strong responses upstream in the olfactory sac had long retention times while odorants that had stronger responses downstream had short retention times. When the air flow was presented in the reverse direction traveling from the internal nares to the external nares, the odorants behaved in

the same way (Mozell, 1964). The odorants with the long retention times had strong responses upstream, which was near the internal nares, and the odorants with the short retention times had strong responses downstream, which was near the external nares. Mozell interpreted these findings to suggest that odorant sorption through the bullfrog's olfactory sac was important for access to the receptors. The odorants with the strong upstream responses were believed to be greatly sorbed upstream with few odor molecules left to be sorbed downstream because of their high sorption strength while the odorants with the stronger downstream responses were believed to be evenly sorbed throughout due to the lower sorption strength. The olfactory sac was hence thought to behave like a chromatographic column.

Mozell *et al*. (1991) further observed that the sorptive properties of odorants interact with the air flow rate in the production of olfactory responses. Mozell characterized sorption by the degree to which the odorant molecules reach their position along the mucosa in accordance with the odorant's physicochemical properties. When the air flow rate is increased, there is a decrease in highly sorbed odorants depositing into the mucosal wall upstream allowing for more of these odorants to travel downstream, which increases the olfactory responses. The weakly sorbed odorants, however, have less time to be

deposited into the mucosal wall when the air flow rate is increased, resulting in somewhat smaller responses.

The rat and human nasal cavities, though more complicated than a straight tube, may also control odorant access according to the same principle of the strength of odorant sorption. The rat nasal cavity consists of a long nonolfactory nasal vestibule, which is where air first enters after coming in through the external nares, and an olfactory region, which is located in the dorsal and posterior section of the nasal cavity. Many branch-like scrolled turbinate structures project from the posterior and lateral walls in the olfactory region of the rat nasal cavity. The olfactory epithelium, which has an aqueous mucus layer, consists of approximately 50% of the nasal cavity surface area in rats (Gross *et al*., 1982). The human nasal cavity, which is less complicated than that of the rat, also has a nonolfactory vestibule where air first enters. The olfactory epithelium is located high in the nasal cavity, predominantly on the dorsal section of the septum and superior turbinate. Only 3% of the total surface area of the human nasal cavity is the olfactory epithelium (Sorokin, 1988).

When the overall air flow rate entering the rat nasal cavity changes, the local air flow rates and patterns in the different spaces of the olfactory epithelium change differentially, contributing to the variations of olfactory responses from these different spaces. The complicated anatomy of the rat nose and the

resulting differential local air flow rates in the different spaces in the nasal cavity greatly affect the responses to odorants of various physicochemical properties. For example, based on the predictions made on the model of the air flow patterns in the rat nasal cavity using the measurements of the cross-sectional areas of the different parts of the airway and other information, (Kimbell *et al*., 1997; Zhao *et al*., 2006; Yang *et al*., 2007a, 2007b; Garcia and Kimbell, 2009), the dorsal-medial region of the rat olfactory epithelium has a greater air flow compared to the lateral region. A greater cross-sectional area has less resistance, which facilitates the entrance of a higher air flow rate and a greater volume of air containing odorant, allowing for greater changes in olfactory responses at different air flow rates compared to the lateral region with lower air flow rates. The lateral region has more surface area and a greater surface area-to-airway volume ratio compared to the dorsal region (Harkema, 1991; Zhao *et al*., 2006). With the differing air velocities, air patterns, and spaces for the odorants to travel, these anatomical variations throughout the olfactory epithelium have critical implications on the odorants' access to receptors and, subsequently, the sensation of the odorants.

When electroolfactogram (EOG) responses were recorded from the intact rat olfactory epithelium (Scott *et al*., 2006), those of the polar and hydrophilic odorants were the most increased by increasing air flow rate and had greater

magnitude in the dorsal-medial region while those of the nonpolar and hydrophobic odorants were little affected by changes in air flow rates and had greater magnitude in the ventral-lateral region. These EOG responses are the result of the interaction between the air flow rate and the distribution of olfactory receptors on the olfactory epithelium. This is similar to the aforementioned observations made by Mozell and colleagues with the chromatographic column model of the olfactory sac of the bullfrog.

These data suggest that the spatial distributions of olfactory responses in the olfactory epithelium correspond to the expression pattern of the olfactory receptors. They also suggest that this expression pattern interacts in accordance with the air flow rate in the nasal cavity for the strategic delivery of odorants to their receptors. This is supported by the data on the expression pattern of olfactory receptor genes. The expression pattern of the rodent olfactory epithelium was originally described as having four zones in an anterior to posterior orientation with specific olfactory receptor genes distributed within one of the zones (Ressler *et al*., 1993; Vassar *et al*., 1993). More recent studies have reported the same general pattern, even though they describe a more continuous arrangement (Iwema *et al*., 2004; Miyamichi *et al*., 2005). The pattern of localization of EOG responses to various odorants as observed from studies performed by Scott and colleagues agree with the receptor gene expression

pattern (Scott *et al*., 1996, 1997, 2000; Scott and Brierley, 1999). The regions of the olfactory epithelium that express the same receptor population show the same responses while the regions that express different receptor populations show different responses. These EOGs were recorded from the medial wall of the exposed olfactory epithelium of an opened rat nasal cavity. Odorants were directly presented onto the epithelium. The EOGs were recorded from the dorsal to ventral areas of the olfactory epithelium in order to obtain responses from all the different receptor expression zones. Because these EOG responses were recorded from an opened nasal cavity without the effects from air flow, they are the result of the inherent properties of the olfactory epithelium. EOG responses recorded from an intact (unopened) nasal cavity, however, are affected by the air flow rates and patterns, which interact with the arrangement of the olfactory receptors along the olfactory epithelium.

These studies of olfactory responses in the olfactory epithelium suggest that the inherent factors, particularly the distribution of olfactory receptors, lead to more sensitivity to the polar, hydrophilic odorants in the dorsal-medial region of the olfactory epithelium where the regional air flow rate is high, and more sensitivity to nonpolar and hydrophobic odorants in the ventral-lateral region where the regional air flow rate is low. The olfactory responses recorded from the intact nasal cavity suggest that the odorants' physicochemical properties

related to sorption (polarity and hydrophilicity/ hydrophobicity) determine how the air flow rate affects the odorants' access to their receptors to generate olfactory responses. There thus appears to be a correspondence between the expression pattern of olfactory receptors and the air flow rate in the governance of the magnitude and localization of the olfactory responses. The EOG responses recorded from the intact nasal cavity, which are influenced by the local air flow rates, therefore represent the imposed properties of the olfactory epithelium. The goal of the present study is to explore the chromatographic column hypothesis in more detail, adding more odorants and adding the condition of multiple pulse odorant stimulation. In order to investigate the issues of retronasal olfaction, and the interactions among the air flow rate, anatomy of the rat nasal cavity, and odorant sorption, I performed several experiments with sixteen odorants of a range of physicochemical properties, in which the odorants were presented to the rat nasal cavity in the orthonasal and retronasal directions. The EOG responses to these odorant stimulations were recorded with the orthonasal responses used to assess the relative retronasal responses. In addition, multiple pulses of retronasal odorant stimulation were presented to the rat nasal cavity in order to simulate mouth movements inside the oral cavity. This procedure was conducted because the sensation of food odorants inside the oral cavity through retronasal olfaction is greatly influenced by mouth

movements, such as masticating and swallowing (Burdach and Doty, 1987). The responses to the multiple pulses of retronasal odorant stimulation may be more complicated than those to the single odorant stimulations. In the analyses, I measured the peak EOG response magnitudes as well as the area under the curve of the EOG responses, which was compared with the peak EOG responses in order to have a more general estimate of the amount of activity reaching the central nervous system. The rat was chosen as the animal model because it is a convenient animal for the current olfaction experiment and has been used in past literature of olfaction research (Buck and Axel, 1991; Vassar *et al*., 1993; Strotmann *et al*., 1994; Zhao *et al*., 1998; Johnson and Leon, 2007). Rats have a highly developed olfactory system and are large enough for surgery and electrophysiology tests.

Methods

The odorants were diluted with mineral oil ranging in ratios from 1:1000 to 1:10. The volume of each odorant dilution was 5 ml. The diluted odorants were stored in tightly covered glass bottles, which were connected with Teflon tubing to the ports of a glass odorant presentation tube during experimental runs. The concentrations of the odorants were tested by a gas chromatograph before the first experimental presentation and again after several experimental presentations. The interval between these tests was three to five days. Sixteen odorants were tested and their abbreviations are found in Table 1. These abbreviations are used in the figures.

Male Sprague-Dawley rats (N = 20) were first injected with atropine (0.01 mg/kg) to prevent the clogging of the nasal passages, and then killed by injection of sodium pentobarbital (100 mg/kg), which allows for minimum bleeding and maximum patency of the airway. A Teflon cannula was placed into the trachea and reached the nasal cavity just above the soft palate (Figure 1A). The cannula was previously tested to ascertain that it does not absorb the odorants itself. For the first set of animals (N = 8), the bones covering the dorsal-medial and ventral-lateral sites of the olfactory epithelium were thinned with a dental burr and removed with fine forceps to uncover the sites for recording (Figure 1B). For the

1 Vinyl Cyclohexane 0.001	CAS no.: 695-12-5
2 Hexanal 0.01	CAS no.: 66-25-1
3 D-Limonene 0.01	CAS no.: 138-86-3
4 Myrcene 0.002	CAS no.: 123-35-3
5 P-Cymene 0.01	CAS no.: 99-87-6
6 Ethyl Butyrate 0.002	CAS no.: 105-54-4
7 Hexanone 0.001	CAS no.: 30637-87-7
8 Isoamyl Acetate 0.001	CAS no.: 123-92-2
9 Hexanoic Acid 0.01	CAS no.: 142-62-1
10 Anisole 0.002	CAS no.: 100-66-3
11 Benzyl acetate 0.01	CAS no.: 140-11-4
12 Heptanol 0.01	CAS no.: 111-70-6
13 D-Carvone 0.01	CAS no.: 99-49-0
14 Phenyl Acetate 0.01	CAS no.: 122-79-2
15 P-tolyl Acetate 0.01	CAS no.: 140-39-6
16 Methyl Benzoate 0.001	CAS no.: 93-58-3

Table 1. List of odorants by odor number (as used in figures) and their dilutions relative to saturation in the air

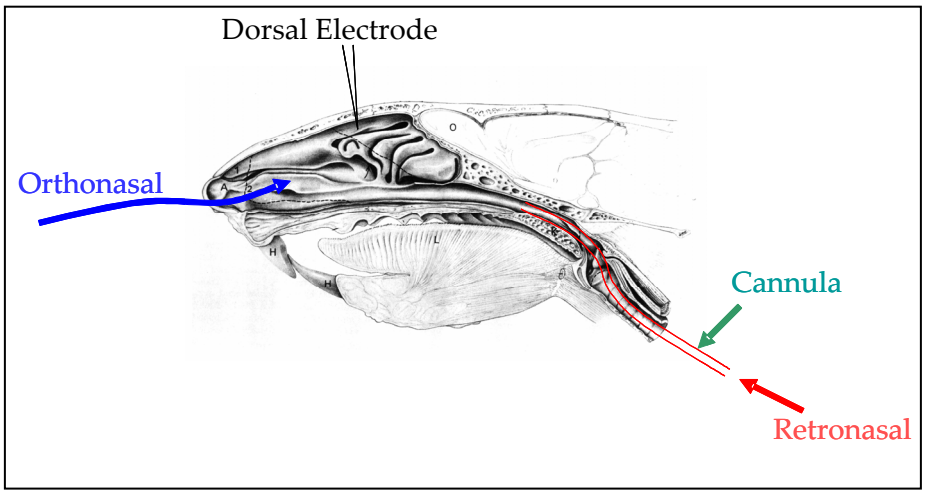

Figure 1A. (Above) The placement of the dorsal-medial electrode and the cannula in the rat as well as the entrances of the odorants in the orthonasal and retronasal directions.

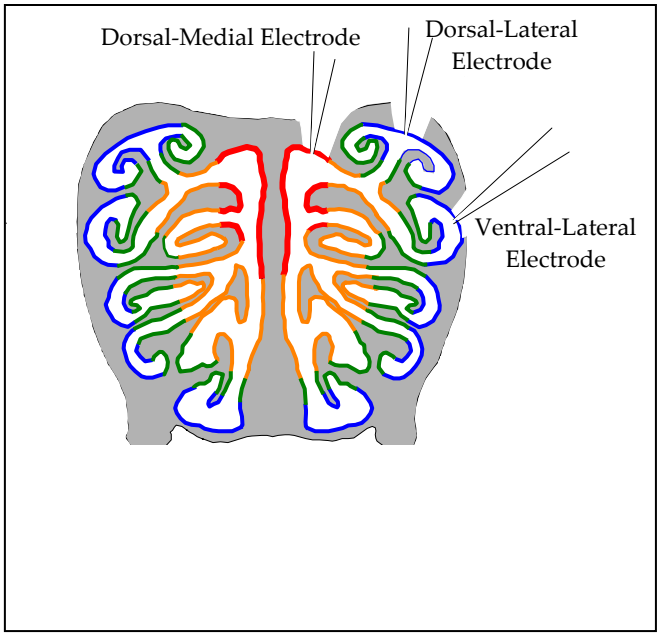

Figure 1B. (Left) The placement of the dorsal-medial, dorsal-lateral, and ventral-lateral electrodes in a coronal cross section of the olfactory epithelium. The different colors represent the different receptor zones of the olfactory epithelium.

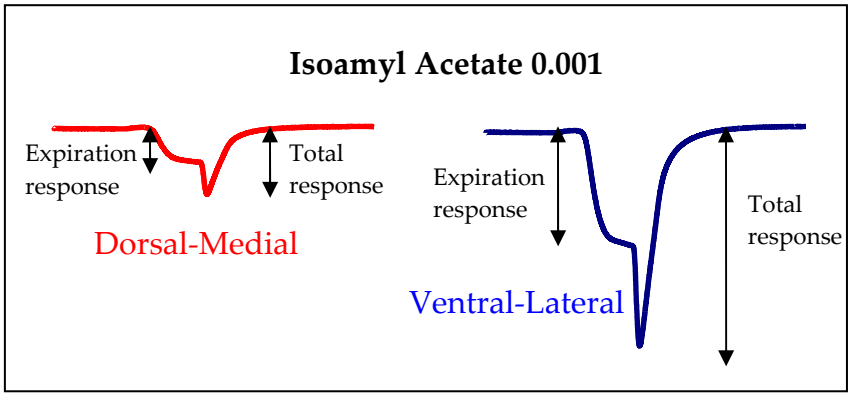

Figure 1C. (Left) An example of a retronasal EOG response with the total and expiration response shown.

second set of animals (N = 12), the same dorsal-medial site and a dorsal-lateral site were used for recording (Figure 1B). Ringer's solution was used to moisturize the two sites of the epithelium throughout an experimental run. A ground in the form of a chlorided silver wire with a ball-shaped end was implanted in EEG paste on the frontal bone of the rat's skull.

Glass electrodes filled with Ringer's solution were used to record the EOG responses. A chlorided silver wire was inserted into each electrode, which was then fitted into a manipulator and lowered into the epithelium. The resistance of the glass electrode tip was <5 MΩ.

Before the actual recording, each electrode was driven through the epithelium to a position that recorded the greatest EOG response to an isoamyl acetate test. A dilution at 0.001 of isoamyl acetate relative to saturated air in mineral oil was used for normalization of the response because it did not evoke significantly different responses on either of the two recording sites in previous experiments (Scott and Brierley, 1999) and because of its intermediate polarity. Recording of EOG responses began with the odorants flowing in either the orthonasal or retronasal directions. The order of the two air flow directions was varied among the animals.

During an experimental run with the odorant stimuli flowing in the orthonasal direction, each odorant was pushed by a clean air flow at 100 ml/min

from the glass bottle to the glass odorant tube, of which the open end was placed in front of the rat's anterior nares. Meanwhile, a humidified and filtered flow of air of 1000 ml/min moved along the odorant tube and throughout the system. These two flow rates further diluted the concentration of each odorant by 0.1. When the odorant stiumuli were set to flow in the retronasal direction, the tubing of the odor bottles was attached to the ports of a different glass odorant tube, which is connected to the posterior end of the cannula. The retronasal air flow rate flowing through the odorant tube and the nasal cavity was 500 ml/min.

The experimental setup had an odorant stimulus duration of 2500 msec per odorant. This non-physiologically long pulse duration was chosen in order to allow for a peak response voltage to occur. For each odor stimulation, a sniff flow rate of 500 ml/min produced by a vacuum put in the cannula was turned on to prevent unfiltered outside air from getting into the rat's nasal cavity. This flow rate corresponds to a moderately, strong sniff as compared to measurement in behaving rats (Youngentob *et al*., 1987). An interval of sixty seconds of clean air flow was imposed between each stimulus to clear out odorant remaining after stimulus presentation. The stimulation of the set of odorants was repeated twice for a total of three presentations per odorant. A computer program written in LabView controlled the odor stimulus sequence and the timing of nasal air flow onsets and offsets. The air flow rates were controlled by manually adjusting a

needle valve resistance, which set the steady state flow rate with a floating ball meter.

During retronasal odorant presentation, an expiratory response is generated. Some odorants also generate an inspiratory response immediately following the expiration upon termination of the odorant stimulation. For the analyses, the retronasal expiration response and the retronasal total response, which consists of the expiration and inspiration responses, were measured (Figure 1C).

During data editing, EOG recordings with noisy tracings, failed responses, large artifacts, or tracings with drifts altering the responses were removed before the analysis. Response size was not a criterion for editing. Measurement of the EOG responses and data editing were performed with a MatLab program written in the lab. Three traces were averaged to represent a response in most cases, although in rare cases only a single trace was used. Comparisons across animals and across odorants were based on these averages. In many cases, there was a small response to a "blank," in which there was air flow, but no odor stimulus. This resulted from electrical artifacts or mechanical movement of the tissue around the electrode or minor odor contamination. These blanks were subtracted. Two measurements were made for each stimulus: the peak negativity and the area under the response curve (the sum of all negative values).

To test the hypothesis that the olfactory responses were related to molecular properties, I compared responses to chemical properties calculated with Molecular Modeling Pro (ChemSW, Fairfield, CA) and to the logarithm of Henry's Law constant (air-water partition coefficient) from the EPA website (https://www.epa.gov/nrmrl/std/cppb/qsar). I used parameters chosen from recent work in our laboratory comparing three datasets describing odor response distribution across the olfactory epithelium (Scott and Sherrill, 2010). That study compared 32 descriptors from Molecular Modeling Pro, including values such as LogP, molecular weight, molecular dimensions, dipole moment and vapor pressure. They proposed an equation based on the Hansen solubility parameter (HSP) and the total electrotopological state (ETS) that correlated strongly (R = 0.92 for the 16 odorants common to the three datasets, R = 0.86 across 86 odorants represented in at least one dataset). The Hansen solubility parameter (Hansen, 2000) estimates the energy necessary for separation of molecules to allow evaporation or solution. The electrotopological state (Kier and Hall, 1999) describes the influences exerted on a molecule's atoms by the charges and distances from the other atoms of that molecule. The equation developed by Scott and Sherrill was -6.108 + 0.267*HSP + 0.071*ETS. The logarithm of Henry's Law constant also correlated strongly with response distribution (R = 0.84), but not as strongly as the variable based on HSP and ETS (which will be referred to

as the HSP-ETS variable). I have calculated and reported the correlation with the logarithm of Henry's Law constant because of its theoretical interest in describing solubility in aqueous media and because of its use in modeling the odorant behavior in the nasal cavity (Zhao *et al*., 2004, 2006).

Results

Orthonasal odorant stimulation

It was important to test whether orthonasal responses to the odors show the same spatial distribution as those of odors previously tested in the lab (Scott *et al*., 1996, 1997, 2000; Scott and Brierley, 1999). The distribution of orthonasal responses was evaluated by plotting the difference between the dorsal-medial and lateral responses normalized to the sum of the dorsal-medial and lateral responses for each odorant. This is analogous to the slope measure used by Scott *et al*. (2000) for measuring responses across the exposed olfactory epithelium. The dorsal-lateral and the ventral-lateral responses were pooled because their slopes were not significantly different based on correlation analyses (see below). Two measurements of the EOG were computed: the peak response and the area under the curve. The peak response was used in previous reports of EOG measurements from the lab. The measurements of the area under the curve tested whether differences in the decay time of the response might affect the estimate of receptor output. In addition, the area under the response curve had been used in reports of nerve activity in frog preparations and their relation to odorant properties (Mozell *et al*., 1991). The plot of the orthonasal dorsal-medial vs. lateral difference values for peak response against the HSP-ETS variable is shown in Figure 2. Testing these correlations for the eight animals with the

ventral-lateral electrode placements vs. the twelve animals with dorsal-lateral electrode placements revealed no significant difference in the slopes of the plot of the HSP-ETS variable vs. orthonasal slope response or the for the retronasal response variables tested later.

The HSP-ETS variable had the highest correlation with the mean values of the normalized orthonasal peak responses (Figure 2, $R = 0.892$). This correlation was higher than that with HSP alone ($F(1, 13) = 12.21$, $P < 0.01$). Nonpolar and water insoluble odorants generally had low values of the variable while polar and water soluble odorants generally had high values. This equation also produced a better correlation with the peak responses than the logarithm of Henry's Law constant ($R = -0.804$), although there is no significant difference between the HSP-ETS variable and the logarithm of Henry's Law constant, as determined from a test of residuals of the two. Polar and water soluble odorants generally had low values of the logarithm of Henry's Law constant while nonpolar and water insoluble odorants had high values, which is the reason that the correlation is in the opposite direction of the calculated equation. Figure 3 shows that a similar relationship with the difference in the orthonasal dorsal-medial and lateral responses as measured by the area under the curve. For this analysis, the correlation between the difference in responses and the HSP-ETS variable was 0.881. (Correlation with logarithm of Henry's Law constant: $R = -$

0.787.) In the subsequent of the various comparisons for retronasal responses, the HSP-ETS variable generally gave the best correlation and I have used that baseline for consistent comparison. All correlations reported were statistically significant at $P < 0.01$.

Retronasal odorant stimulation

Figure 4 displays the relationship between the normalized retronasal responses and the HSP-ETS variable. Responses to particular odorants presented retronasally occurred in the same parts of the epithelium as when those odorants were presented in the orthonasal direction. However, for some odorants, those responses were substantially reduced for retronasal presentation. To summarize these effects, I have summed the dorsal-medial and lateral responses to look at the overall effect of retronasal presentation. The sum of retronasal responses to each odorant was divided by the sum of the orthonasal responses to that odorant. The red line in Figure 4 represents only the expiratory response from the retronasal stimulation. The blue line represents the total response, which is the combined the expiration and the inspiration responses. With correlation coefficients of $R = -0.87$ for the former and $R = -0.845$ for the latter when each are compared with the HSP-ETS variable, the two types of retronasal responses show very similar relationships with the molecular properties of the odorants.

This is despite the inspiration responses included in some of the odorants' total responses.

Three pulses odorant stimulation

Three pulses of odorant stimuli were presented during orthonasal and retronasal flow in nineteen animals in an attempt to more nearly mimic natural sniffing. The orthonasal EOG area responses to the triple pulses of stimuli were normalized to the area of the orthonasal responses and plotted the same way as the responses to the single pulse of stimuli. The correlations between the area under the curve and the HSP-ETS variable (Figure 5, $R = 0.849$) show similar relationships as compared to those of the responses to the single pulse of odorants. (Correlation with logarithm of Henry's Law constant: $R = -0.727$.) The retronasal EOG responses to the three pulses of odor stimulation were only computed for their total response including the inspiration and expiration because inspiration and expiration response curves were frequently difficult to distinguish. These retronasal area responses to the three pulses of odor stimulation were normalized the same way as those to the single pulse of orthonasal odor stimulation. The correlation between the retronasal area under the curve and the HSP-ETS variable was $R = -0.803$ Figure 6. (Correlation with logarithm of Henry's Law constant: $R = 0.729$.)

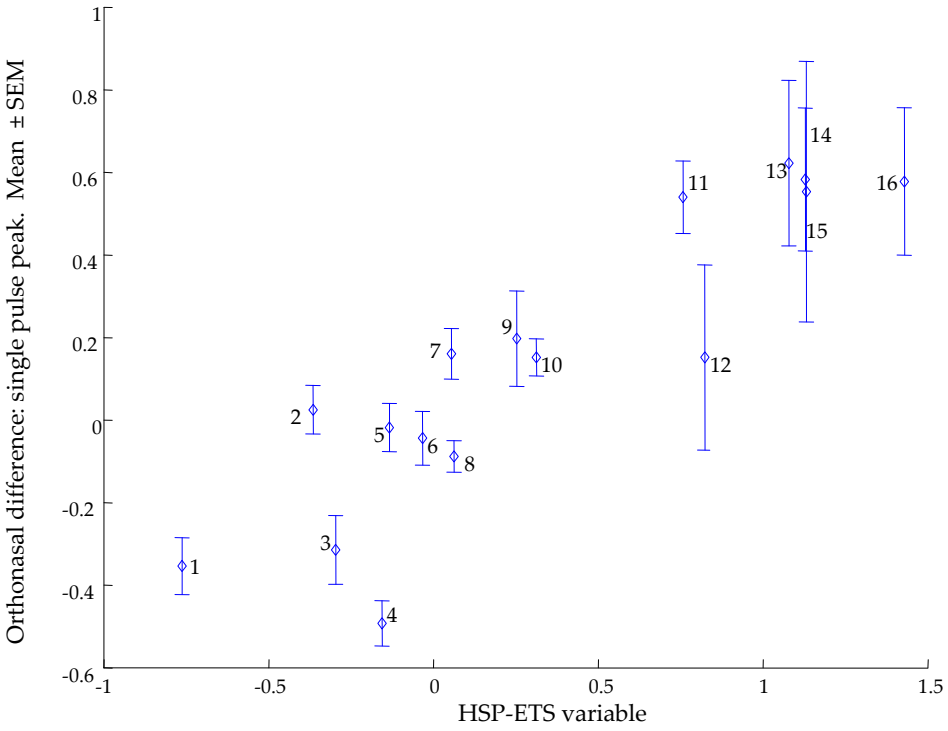

Figure 2. The mean and standard error (N = 20) of the difference in orthonasal peak response to sixteen odorants between the dorsal-medial and lateral positions divided by the sum response of the two.

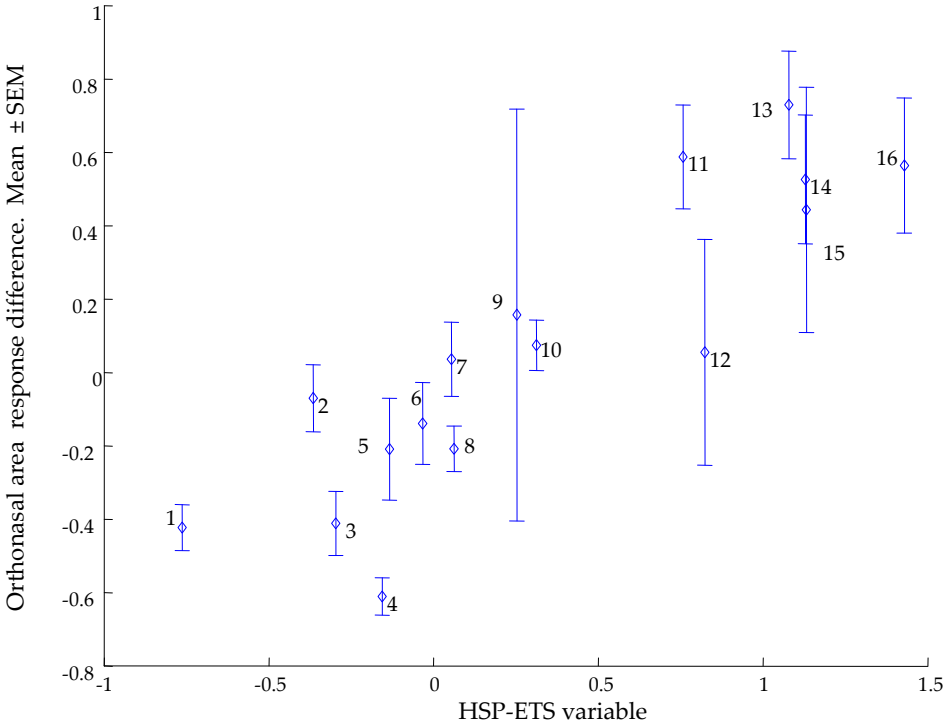

Figure 3. shows similar relationship with the difference in the orthonasal dorsal-medial and lateral responses as measured by the area under the curve as the relationship from Figure 2.

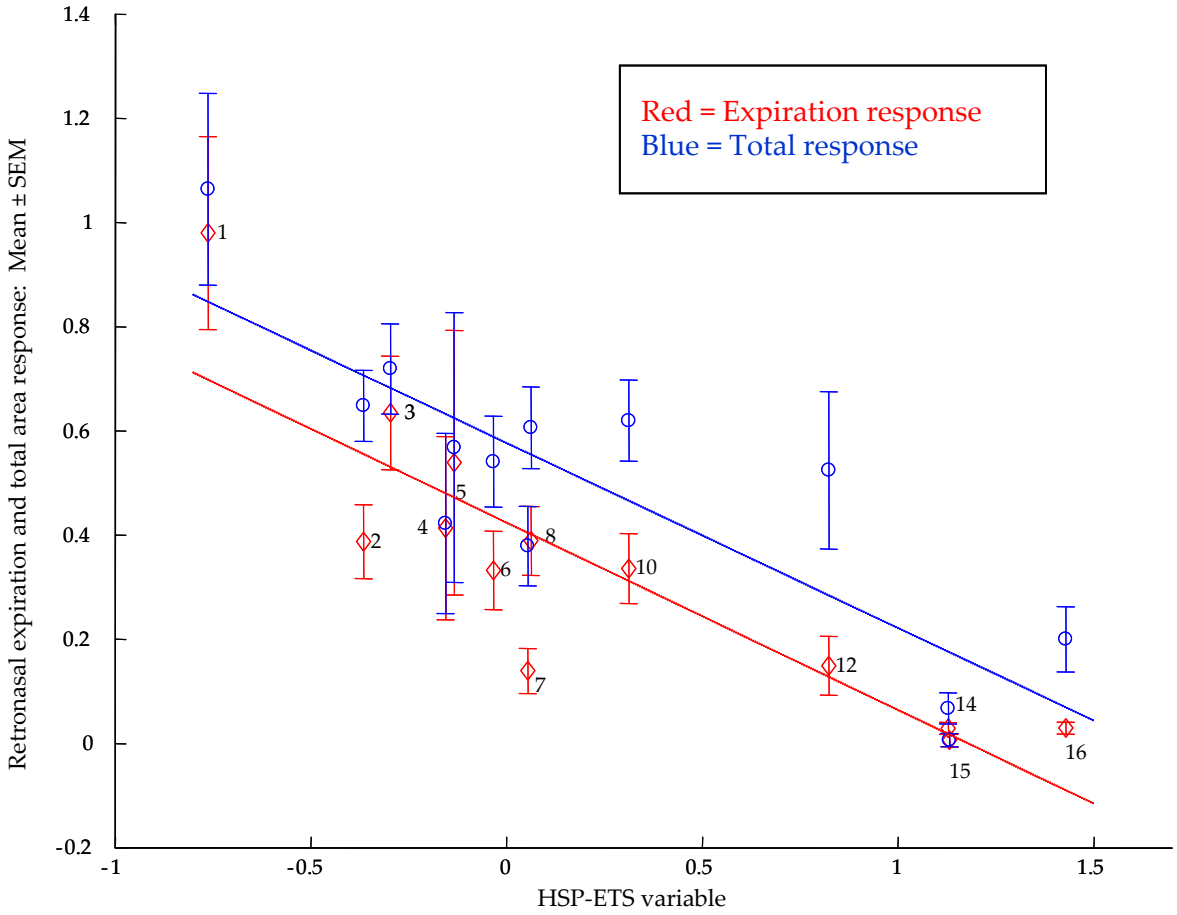

Figure 4. The retronasal expiration response (in red) and the retronasal total response (in blue) have similar relationships with the HSP-ETS variable.

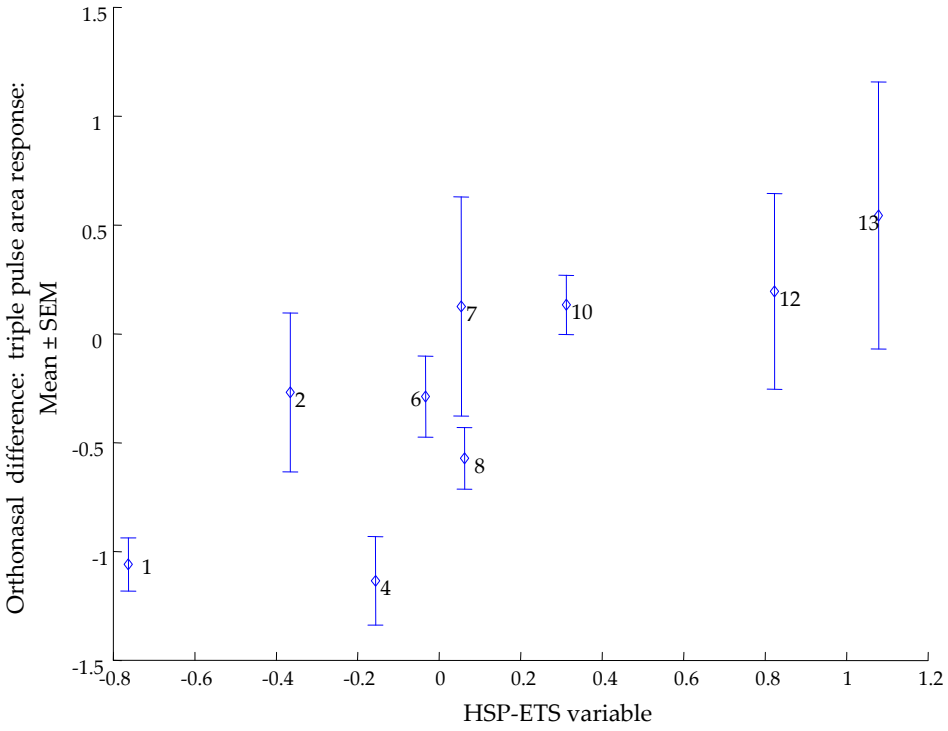

Figure 5. shows the relationship between the normalized difference of the orthonasal dorsal-medial and lateral area responses under the curve to three pulses of odorant stimulation.

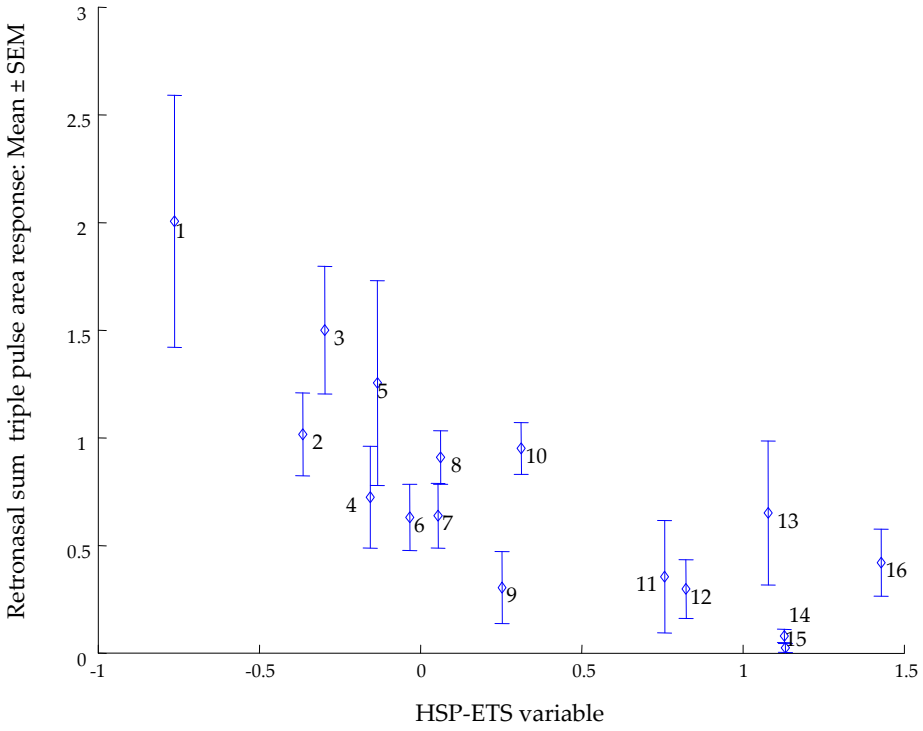

Figure 6. shows the relationship between the normalized sum of the retronasal dorsal-medial and lateral area responses under the curve to three pulses of odorant stimulation.

Discussion

Throughout the study, the terms polarity, hydrophobicity, and hydrophilicity were used to describe the physicochemical properties of the odorants. These are not perfect descriptors of the HSP-ETS variable. These terms are merely convenient descriptors and do not capture all the molecular characteristics that affect the odorants' responses. On the other hand, the Henry's Law constant is generally recognized as a good descriptor of the air-water partition.

The EOG responses to the odorants are different during orthonasal and retronasal flow. When odorants travel within the nasal cavity in the orthonasal direction, the responses to the polar odorants are the greatest in the dorsal-medial region of the olfactory epithelium while the responses to many nonpolar odorants are the greatest in the lateral region. When odorants travel within the nasal cavity in the retronasal direction, however, the responses to the odorants are greatly reduced compared to responses of odorants flowing in the orthonasal direction. The nonpolar odorants are the most effective stimuli during the expiration phase of retronasal odor presentation, but the polar odorants elicited much smaller responses for both the dorsal-medial and lateral regions of the olfactory epithelium. Overall, the relationships between the different responses measured and the HSP-ETS variable were similar. The relationship between the

HSP-ETS variable and the degree of response was strong regardless of whether there was one or three pulses of odor stimulation or whether the peak response or area response was measured. For example, the response peaks and the area responses under the curve, the single and triple pulse odorant stimulation, and the retronasal expiration response and retronasal total response are all pairs with similar relationships and high correlations.

Kurtz *et al*. (2004) and Mozell and Hornung (1985) had attempted to compare mucosal solubility with water solubility. The mucosal solubility, however, is influenced by other factors, such as odorant binding proteins in the mucus. Henry's Law constant describes the equilibrium between the air and water phases. It has been used previously by Kurtz *et al*. (2004) to measure odorant solubility in the nasal mucosa. The logarithm of Henry's Law constant is a good descriptor of the relationship between the physicochemical properties of the odorants and the spatial distribution of the odorants' responses along the olfactory epithelium. Compared with the HSP-ETS variable, it does not have as high correlations with the normalized responses of the odorants, but the correlations of the two variables are not significantly different.

The EOG was used as a measure of the odorant responses because this recording technique has several advantages. It has a simple preparation. Responses can be collected from recently killed animals, which have an

advantage in that it is easier to maintain the patency of the airway in dead animals over live animals. It allows for the recording of a population of cells instead of only a single cell, which is useful because the voltage responses represent a group of neurons instead of only one. By summing EOGs from two very different regions of the olfactory epithelium, we are able to give a reasonable estimate of information entering the olfactory bulb.

The area under the curve for the EOG responses to the multiple pulses of odorant stimulation was calculated because different odorants generate differently shaped response curves, particularly the size of the modulations. This is similar to the modulations of glomerular responses to various odorants (Spors *et al*., 2006). This would affect the temporal summation in multiple sniffs. The nonpolar odorants, such as vinyl cyclohexane, have low modulations while the polar odorants have high modulations. The area under the response curve would not necessarily be expected to correlate with the magnitude of the peak response, however, both measures correlate strongly with the molecular descriptors we have used. This shows that the molecular properties of the odorants really are important factors to the distribution of responses on the olfactory epithelium as well as the magnitude of the orthonasal and retronasal responses.

One limitation of the current study was the duration of odorant stimulation of 2500 ms is a nonphysiologically long pulse duration. It was selected because the EOG responses would require at least one second to reach their peaks during orthonasal flow (Scott *et al*., 2006). Exploratory experiments were performed using different time durations, but not enough odorants were tested or experiments completed in order to make comparisons. Since the responses during long-duration presentation greatly resemble those recorded by direct application of odorant to an exposed epithelium of an opened nasal cavity (Scott-Johnson *et al*., 2000), the long-duration responses are good indicators of maximal access of the odorants to the olfactory epithelium. The data from the current study suggests that the nonpolar odorants are able to obtain near maximal potential of access to their olfactory receptors during retronasal flow.

The air flow patterns in the rat nasal cavity appear to work in combination with the sorptive properties of the odorants in efficiently transporting the odorants to their receptors. The inspiratory flow travels through the dorsal olfactory region, turns ventrally, then turns rostrally towards the anterior nares, and finally reverses direction again before leaving through the nasopharynx (Kimbell *et al*., 1997; Zhao *et al*., 2006; Yang *et al*., 2007). The data suggests that the variables HSP-ETS and the logarithm of Henry's Law constant correlates with the degree of sorption. During orthonasal flow, there is high air velocity in

the olfactory cleft, favoring odorants that are highly sorbed, producing great responses in the dorsal-medial epithelium. During retronasal flow, there is low air velocity, favoring odorants that are weakly sorbed to produce greater responses.

The expiratory air flow stream is more of a straight line from the nasopharynx to the anterior nares. The small retronasal responses to the polar, hydrophilic odorants compared to their much greater orthonasal responses may be due to decreased concentration of these odorants during retronasal flow. The decreased concentration may be related to the differential air flow rates in the spaces of the nasal cavity where the expiratory air streams exist. Lowered air flow rates would allow for more residence time in the nasal cavity for the polar odorants to be sorbed. The lowered air flow rates, however, may favor strong responses from the nonpolar, hydrophobic odorants as the odorants are not highly sorbed onto the walls of the nasal cavity (Mozell, 1991).

Upon termination of the long duration retronasal odorant stimulation, an inspiratory response is observed for some odorants. The inspiratory response is particularly large for odorants of intermediate polarity (such as isoamyl acetate) and did not exist for very polar (such as methyl benzoate) or very nonpolar (such as vinyl cyclohexane) odorants. The inspiratory response may be a result of odor near the front of the nasal cavity being drawn into the olfactory region during

inspiration. It does not occur frequently for the nonpolar and hydrophobic odorants because these odorants have already elicited a maximum retronasal (expiratory) response as they do not easily sorb out of the air stream. It also does not occur frequently for the polar and hydrophilic odorants because the concentration of these odorants in the air stream is low as they are easily adsorbed onto the nasal mucosa. Since the nonpolar odorants infrequently generated an inspiration response, which is a combination of the expiratory and inspiratory responses, their total retronasal response often comprised of only the expiration response.

The odors in the natural environment of humans and animals are complex mixtures of up to hundreds of compounds. When mixtures of odorant compounds enter the nasal cavity, the different odorant compounds are hypothesized to be differentially sorbed onto the nasal mucosa. The degree and location of sorption in the nasal cavity are dependent on the individual compound's physicochemical properties, the direction of air flow, and the air flow rate. Consequently, when a mixture is presented in retronasal flow, the polar compounds will reach the olfactory epithelium in a lower concentration than when presented flow orthonasal flow and the sensations are likely to be perceived differently.

The data from these proposed experiments can contribute to the food industry to create healthier and more palatable foods. The odorants with the greatest retronasal expiratory EOG responses were the nonpolar, hydrophobic odorants. Since fat-related odors are likely to be hydrophobic, their delivery to the olfactory region is favored during retronasal flow over other odors. This has implications in the development of palatable and healthful foods. The odorants with intermediate polarity and solubility had the largest total expiratory and inspiratory responses during retronasal odorant delivery, and are perhaps also critical in flavor sensation. For example, knowing which odorants of healthy fats (e.g., monounsaturated and polyunsaturated fats) produce the greatest responses (i.e., generate the greatest olfactory sensations) can be helpful for the food industry so that it can select these healthy fats as ingredients in their foods to make them more flavorful without having to use the unhealthy saturated or *trans* fats. My study may also be interesting in helping the food industry choose flavors with the desired magnitude of olfactory stimulation to manufacture a healthy food product with a pleasurable flavor sensation.

References

1. Badonnel K., Durieux D., Monnerie R., Grébert D., Salesse R., Caillol M., and Baly C. (2009) Leptin-sensitive OBP-expressing mucous cells in rat olfactory epithelium: a novel target for olfaction-nutrition crosstalk? *Cell and Tissue Research*. 338.1: 53-66.
2. Beidler L.M., and Smith J.C. (1991) Effects of radiation therapy and drugs on cell turnover and taste. Getchell T.V., Doty R.L., Bartoshuk L.M., and Snow J.B.J., eds. *Smell and taste in health and disease.* New York: Raven Press, 753-763.
3. Bergdahl M. and Bergdahl J. (2002) Perceived taste disturbance in adults: prevalence an association with oral and psychological factors and medication. *Clinical Oral Investigations*. 6: 145-149.
4. Berteretche M.V., Dalix A.M., d'Ornano A.M.C., Bellisle F., Khayat D., and Faurion A. (2004) Decreased taste sensitivity in cancer patients under chemotherapy. *Support Care Cancer*. 12: 571-576.
5. Buck L.B. and Axel R. (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell*. 65.1: 175-187.

6. Burdach K.J., and Doty R.L. (1987) The effects of mouth movements, swallowing, and spitting on retronasal odor perception. *Physiology & Behavior*. 41: 353-356.
7. Chauncey H.H., Muench M.E., Kapur K.K., and Wayler A.H. (1984) The effect of the loss of teeth on diet and nutrition. *International Dental Journal*. 34: 98-104.
8. de Jong N., Mulder I., de Graaf C., and van Staveren W.A. (1999) Impaired sensory functioning in elders: the relation with its potential determinants and nutritional intake. *The Journals of Gerontology*. 54A.8: B324-B331.
9. du Toit D.F. (2003) The tongue: structure and function relevant to disease and oral health. *SADJ*. 59.9: 375-383.
10. Duffy V.B. (2007) Variation in oral sensation: implications for diet and health. *Current Opinion in Gastroenterology*. 23.2: 171-177.
11. Fukunaga A., Uematsu H., and Sugimoto K. (2005) Influences of aging on taste perception and oral somatic sensation. *The Journals of Gerontology*. 60A.1: 109-113.
12. Garcia G.J.M. and Kimbell J.S. (2009) Deposition of inhaled nanoparticles in the rat nasal passages: Dose to the olfactory region. *Inhalation Toxicology*. 21.14: 1165-1175.

13. Gross E.A., Swenberg J.A., Field S., and Popp J.A. (1982) Comparative morphometry of the nasal cavity in rats and mice. *Journal of Anatomy*. 135: 83-88.

14. Hansen C.M. (2000) *Hansen Solubility Parameters*. Hoersholm: CRC Press.

15. Harkema J.R. (1991) Comparative aspects of nasal airway anatomy: relevance to inhalation toxicology. *Toxicologic Pathology*. 19.4: 321-336.

16. Heilmann S. and Hummel T. (2004) A new method for comparing orthonasal and retronasal olfaction. *Behavioral Neuroscience*. 118.2: 412-419.

17. Iwema C.L., Fang H., Kurtz D.B., Youngentob S.L., Schwob J.E. (2004) Odorant receptor expression patterns are restored in lesion-recovered rat olfactory epithelium. *Journal of Neuroscience*. 24.2: 356-69.

18. Johnson B.A. and Leon M. (2007) Chemotopic odorant coding in a mammalian olfactory system. *The Journal of Comparative Neuroology*. 501: 1: 1-34.

19. Kier L.B. and Hall L.H. (1999) *Molecular Structure Description: The Electrotopological State*. San Diego: Academic Press.

20. Kimbell J.S., Godo M.N., Gross E.A., Joyner D.R., Richardson R.B., and Morgan K.T. (1997) Computer simulation of inspiratory airflow in all

regions of the F344 nasal passages. *Toxicology and Applied Pharmacology*. 145: 388-398.

21. Ko H.J., Lee S.H., Oh E.H., and Park T.H. (2010) Specificity of odorant-binding proteins: a factor influencing the sensitivity of olfactory receptor-based biosensors. *Bioprocess and Biosystems Engineering*. 33.1: 55-62.
22. Kurtz D.B., Zhao K., Hornung D.E., and Scherer P. (2004) Experimental and numerical determination of odorant solubility in nasal and olfactory mucosa. *Chemical Senses*. 29: 763-773.
23. Landis B.N., Giger R., Ricchetti A., Leuchter I., Hugentobler M., Hummel T., and Lacroix J.S. (2003) Retronasal olfactory function in nasal poypsis. *Laryngoscope*. 113.11: 1993-1997.
24. Linforth R., Martin F., Carey M., Davidson J., and Taylor A.J. (2002) Retronasal transport of aroma compounds. *Journal of Agricultural and Food Chemistry*. 50: 1111-1117.
25. Miyamichi K., Serizawa S., Kimura H.M., and Sakano H. (2005) Continuous and overlapping expression domains of odorant receptor genes in the olfactory epithelium determine the dorsal/ventral positioning of glomeruli in the olfactory bulb. *Journal of Neuroscience*. 25.14: 3586-3592.

26. Mozell M.M. (1964) Evidence for sorption as a mechanism of the olfactory analysis of vapors. *Nature (London)*. 203: 1336.

27. Mozell M.M. (1970) Evidence for a chromatographic model of olfaction. *The Journal of General Physiology*. 56: 46-63.

28. Mozell M.M. and Hornung D.E. (1985) Peripheral mechanisms in the olfactory process. In Pfaff D. (ed.), *Taste, Olfaction, and the Central Nervous System*. The Rockefeller University Press, New York, 253-297.

29. Mozell M.M. and Jagodowicz M. (1973) Chromatographic separation of odorants by the nose: retention times measured across *in vivo* olfactory mucosa. *Science*. 181: 1247-1249.

30. Mozell M.M., Kent P.F., and Murphy S.J. (1991) The effect of flow rate upon the magnitude of the olfactory response differs for different odorants. *Chemical Senses*. 16.6: 631-649.

31. Ng K., Woo J., Kwan M., Sea M., Wang A., Lo R., Chan A., and Henry C.J.K. (2004) Effect of age and disease on taste perception. *Journal of Pain and Symptom Management.* 28.1: 28-34.

32. Ohno T., Uematsu H., Nozaki S., and Sugimoto K. (2003) Improvement of taste sensitivity of the nursed elderly by oral care. *Journal of Medical and Dental Sciences*. 50: 101-107.

33. Ressler K.J., Sullivan S.L., and Buck L.B. (1993) A zonal organization of odorant receptor gene expression in the olfactory epithelium. *Cell*. 73: 597-609.

34. Ritchie C.S. (2002) Oral health, taste, and olfaction. *Clinics in Geriatric Medicine*. 18: 709-717.

35. Rozin P. (1982) Taste-smell confusions and the duality of the olfactory sense. *Perception & Psychophysics*. 31: 397–401.

36. Schiffman S.S. (1993) Perception of taste and smell in elderly persons. *CRC critical reviews in food science and nutrition*. 33: 17-26.

37. Scott J.W. (2006) Sniffing and spatiotemporal coding in olfaction. *Chemical Senses*. 31: 119-130.

38. Scott J.W., Acevedo H.P., and Sherrill L. (2006) Effects of concentration and sniff flow rate on the rat electroolfactogram. *Chemical Senses*. 31: 581-593.

39. Scott J.W., Acevedo H.P., Sherrill L.K., and Phan M. (2007) Responses of the rat olfactory epithelium to retronasal air flow. *Journal of Neurophysiology*. 97: 1941-1950.

40. Scott J.W. and Brierley T. (1999) A functional map in rat olfactory epithelium. *Chemical Senses*. 24: 679-690.

41. Scott J.W., Brierley T., and Schmidt F.H. (2000) Chemical determinants of the rat electroolfactogram. *Journal of Neuroscience*. 20: 4721-4731.

42. Scott J.W., Shannon D.E., Charpentier J., Davis L.M., and Kaplan C. (1997) Spatially organized response zones in rat olfactory epithelium. *Journal of Neurophysiology*. 77: 1950-1962.

43. Scott J.W. and Sherrill L.K. (2010) Chemical determinants of rat olfactory epithelium response. Poster presented at: 2010 Association for Chemoreception Sciences Meeting. April 2010: St. Petersburg, Florida.

44. Scott-Johnson P.E., Blakley D., and Scott J.W. (2000) Effects of air flow on rat electroolfactogram. *Chemical Senses*. 25.6: 761-768.

45. Ship, J.A. The influence of aging on oral health and consequences for taste and smell. *Physiology & Behavior*. 66.2 (1999): 209-215.

46. Ship, J.A., Duffy V., Jones J., and Langmore S. (1996) Geriatric oral health and its impact on eating. *Journal of the American Geriatrics Society*. 44.4: 456-464.

47. Ship J.A. and Weiffenbach J.M. (1993) Age, gender, medical treatment, and medication effects on smell identification. *The Journals of Gerontology. Series A, Biological sciences and medical sciences*. 48: M26-M32.

48. Small D.M., Gerber J.C., Mak Y.E., and Hummel T. (2005) Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans. *Neuron*. 47: 593-605.

49. Sorokin S.P. (1988) The respiratory system. In Weiss L. (ed.), *Cell and Tissue Biology: A Textbook of Histology*. Urban & Schwarzenberg, Inc., 751-814.

50. Spors H., Wachowiak M., Cohen L.B., and Friedrich R.W. (2006) Temporal dynamics and latency patterns of receptor neuron input to the olfactory bulb. *Journal of Neuroscience*. 26.4: 1247-59.

51. Strotmann J, Wanner I, Helfrich T, Beck A, and Breer H. (1994) Rostro-caudal patterning of receptor-expressing olfactory neurones in the rat nasal cavity. *Cell and Tissue Research*. 278.1: 11-20.

52. Taylor A.J. (1996) Volatile flavor release from foods during eating. *Critical Reviews in Food Science and Nutrition*. 36.8: 765-784.

53. Vassar R., Ngai J., and Axel R. (1993) Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. *Cell*. 74.2: 309-318.

54. Waylar A.H., Perlmuter L.C., Cardello A.V., Jones J.A., and Chauncey H.H. (1990) Effects of age and removable dentition on taste. *Special Care in Dentistry*. 10: 107-113.

55. Weiffenbach J.M., Cowart B.J., and Baum B.M. (1986) Taste intensity perception in aging. *The Journals of Gerontology*. 41: 460-468.

56. Yang G.C., Scherer P.W., Zhao K., and Mozell M.M. (2007) Numerical modeling of odorant uptake in the rat nasal cavity. *Chemical Senses*. 32.3: 273-84.

57. Yang G.C., Scherer P.W., and Mozell M.M. (2007) Modeling inspiratory and expiratory steady-state velocity fields in the Sprague-Dawley rat nasal cavity. *Chemical Senses*. 32.3: 215-23.

58. Youngentob S.L., Mozell M.M., Sheehe P.R., and Hornung D.E. (1987) A quantitative analysis of sniffing strategies in rats performing odor detection tasks. *Physiology & Behavior*. 41: 59-69.

59. Zhao H., Ivic L., Otaki J.M., Hashimoto M., Mikoshiba K., and Firestein S. (1998) Functional Expression of a Mammalian Odorant Receptor. *Science*. 279.5348: 237-242.

60. Zhao K., Dalton P., Yang G.C., and Scherer P.W. (2006) Numerical modeling of turbulent and laminar airflow and odorant transport during sniffing in the human and rat nose. *Chemical Senses*. 31: 107-118.

61. Zhao K., Scherer P.W., Hajiloo S.A., and Dalton P. (2004) Effect of anatomy on human nasal air flow and odorant transport patterns: implications for olfaction. *Chemical Senses*. 29.5: 365-379.