Chem. Senses 31: 119–130, 2006
doi:10.1093/chemse/bjj013
Advance Access publication December 14, 2005

Sniffing and Spatiotemporal Coding in Olfaction

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Abstract

The act of sniffing increases the air velocity and changes the duration of airflow in the nose. It is not yet clear how these changes interact with the intrinsic timing within the olfactory bulb, but this is a matter of current research activity. An action of sniffing in generating a high velocity that alters the sorption of odorants onto the lining of the nasal cavity is expected from the established work on odorant properties and sorption in the frog nose. Recent work indicates that the receptor properties in the olfactory epithelium and olfactory bulb are correlated with the receptor gene expression zones. The responses in both the epithelium and the olfactory bulb are predictable to a considerable extent by the hydrophobicity of odorants. Furthermore, receptor expression in both rodent and salamander nose interacts with the shapes of the nasal cavity to place the receptor sensitivity to odorants in optimal places according to the aerodynamic properties of the nose.

Key words: electroolfactogram, flow rate, odorant hydrophobicity, sorption

Introduction

A sniff is a change in the rate and/or volume of airflow through the nose, apparently for the purpose of improving olfactory detection. This review will try to summarize how this change in airflow may interact with the spatial distribution of response in the vertebrate olfactory epithelium and olfactory bulb. Along the way it will point out a number of unresolved issues. The other reviews in this series will likely comment more specifically on temporal processing in the olfactory bulb, on the motor control of sniffing, and on the airflow patterns in the nose. However, some remarks on these issues are important to the focus of my comments, so I will begin with them.

What does a sniff accomplish?

When a terrestrial vertebrate encounters an odor it tends to change its respiratory pattern to more frequent inspirations of apparently greater air velocity. This is particularly well documented for rodents, which adopt this pattern for almost any novel stimulus and do not depend on olfaction for its initiation (Welker, 1964). There are a number of reports that document the changes in frequency of sniffing (Welker, 1964; Komisaruk, 1970; Macrides *et al*., 1982), but unfortunately only one (Youngentob *et al*., 1987) has studied the changes in flow rate. Youngentob *et al*. (1987) found that the mean flow of a maximum inspiratory sniff for a trained animal trying to detect a weak odor (12.5 ml/s) is well above the average of all sniffs recorded when the animal was detecting a stronger odor (4.8 ml/s). Unfortunately, they were not able to apply the same techniques to quiet breathing. The flow rates during quiet breathing found in the literature are based on dividing tidal volume by the inspiratory time. They vary widely from values below to values above those found by Youngentob *et al*. (1987) for sniffing (Walker *et al*., 1997; Ohtake *et al*., 1998; Zhang and Bruce, 1998; Stephenson *et al*., 2001; Seifert and Mortola, 2002). This may be due in part to variations in the definition of the inspiration time. Inspiratory flow rate increases with breathing frequency (Walker *et al*., 1997) and is higher in wakefulness than in rapid eye movement or nonrapid eye movement sleep (Stephenson *et al*., 2001). The absence of good information on changes in flow rate from quiet breathing to active sniffing makes it very difficult to evaluate the behavioral and aerodynamic significance of sniffing. While it is obvious that sniffing does change flows in the nose, the degree of the change is an important unresolved question in the field.

Does the brain correlate processing with the sniff?

The behavioral studies of sniffing have suggested that there is an active process modulating olfactory bulb responses during sniffing. This active nature is indicated by substantial

literature on the relation between sniffing and hippocampal theta rhythm (Komisaruk, 1970; Macrides, 1975; Macrides *et al.*, 1982; Kay, 2005), by recordings in olfactory bulb slices showing that the olfactory bulb circuitry tends to resonate at the frequencies of the theta rhythm (Schoppa and Westbrook, 2001; Margrie and Schaefer, 2003; Balu *et al.*, 2004), and by evidence that human sniffs are actively modulated (Sobel *et al.*, 1998; Johnson *et al.*, 2003). Several human investigators have proposed using the feedback onto the motor control of sniffing as an assay for olfactory function in clinical situations (Kendal-Reed *et al*, 1998; Frank *et al.*, 2003). Sobel *et al.* (2000) showed that regulation of duration could take place during a sniff in order to improve odor detection. It would be important to know whether the mechanisms generating the sniff feedback via the centrifugal circuitry to the olfactory bulb.

Sniffing may produce optimal temporal sequences in the olfactory bulb. The olfactory bulb responds transiently to odor stimulation. Long-duration odor stimuli produce sequences of bursts and pauses in the spike pattern that often outlasts the odor stimulus pulse (Kauer and Shepherd, 1977; Harrison and Scott, 1986; Wellis *et al.*, 1989; Duchamp-Viret and Duchamp, 1997). These patterns persist even when the stimulation rate is varied artificially (Macrides, 1977). Repetitive stimulation at the rates of normal breathing produce sustained responses that last for many cycles (Chaput and Panhuber, 1982). Recordings in olfactory bulb slices have shown evidence for oscillations at frequencies near the breathing and sniff rates. Electrical stimulation evokes oscillations at frequencies around 2 Hz in mitral cells (Schoppa and Westbrook, 2001). A population of external tufted cells exhibits spontaneous burst frequencies in the range of 0.5–8.8/s (McQuiston and Katz, 2001; Hayar *et al.*, 2004). One likely interpretation is just that the bulb is passively tuned to respond best to frequencies of input that are a little faster than normal breathing. Alternatively, there may be active processes associated with the sniff that might modulate bulb activity through centrifugal systems known to project into the bulb (Nakashima *et al.*, 1978; Davis and Macrides, 1981; Luskin and Price, 1983; Jiang *et al.*, 1996; Paolini and McKenzie, 1996, 1997a,b). These have not been extensively investigated with respect to the timing beyond the observations of Nickell and Shipley (1988) that there is substantial potential of the effects of horizontal nucleus of the diagonal band stimulation at frequencies around 10 Hz and the study by Young and Wilson (1999) of the inhibitory patterns evoked with electrical stimulation of the olfactory tract at 1–5 Hz. Of course, the action of theta locking may not be exclusively in the olfactory bulb but could also involve the terminal areas like piriform cortex. Linster *et al.* (1999) have shown that the diagonal band of Broca can modulate the piriform cortex as well as the olfactory bulb, so understanding of the timing of sniff behavior may require the study of both.

Recordings from the bulb have not indicated differences between operations at frequencies near those for quiet breathing (nearly 2/s for most of the studies noted earlier) versus the faster frequencies noted in active sniffing. Welker (1964) reported sniffing rates of 6–10/s. Macrides *et al.* (1982) suggest 4–8/s. The durations from Youngentob *et al.* (1987) are consistent with about 7/s. There is a methodological division among researchers studying the olfactory bulb *in vivo* with respect to the use of an ''artificial sniff'' produced by a retronasal cannula (Macrides and Chorover, 1972; Wellis *et al.*, 1989; Imamura *et al.*, 1992) or a ''freely breathing'' preparation with the flow in the nose tied to breathing (Chalansonnet and Chaput, 1998; Buonviso *et al.*, 2003; Cang and Isaacson, 2003). Some reports show spontaneous activity correlated with respiration independent of airflow in the nose (Ravel *et al.*, 1987; Ravel and Pager, 1990), although Philpot *et al.* (1997) report that obstructing the naris removed such correlation for all but two of 38 cells. Sobel and Tank (1993) found that there was no difference between the response pattern to saturated amyl acetate odor when airflow through the nose was synchronized with breathing or not synchronized. The artificial sniff preparation offers some advantages that have not been explored extensively. Aside from Macrides' (1977) observations over a narrow range of frequencies (1–3 Hz) very little experimental manipulation of the sniff rate has been performed. These observations could certainly be extended to test whether there is a change in olfactory information transmission at the frequencies of quiet breathing versus those of exploratory sniffing. Some of these issues are discussed in more detail by Buonviso *et al.* (2005).

How does changing stimulus flow rate in the nose affect processing?

There are several important issues in this question. The first is the well-developed set of observations suggesting that terrestrial vertebrate noses act to separate odorants by chemical properties in a flow-dependent manner, the so-called chromatographic process in olfaction. Another issue is the observation of spatial distribution of odor responses in the epithelium and its correlation with the expression pattern of olfactory receptors. Finally, what are the airflow rates in the nose and how do they interact with the chromatographic process?

Flow rate and receptor response

Beginning with the observations of Mozell (1964, 1966) on frog olfactory epithelium, there have been a series of papers demonstrating differential sorption of odorants on the olfactory epithelium. This was demonstrated by comparing responses from axons innervating regions near the internal and external nares of the frog to show that the relative response size of response to these odors depends on direction and on the physical properties of the odorant. He proposed that response depended to a considerable extent on the degree to which odorants were removed from the air into the epithelial lining of the nose. Subsequent experiments showed

that the physiological responses could be substantially predicted by the retention times of these odorants on a polar gas chromatograph column (Mozell, 1970; Mozell and Jadodowicz, 1973). Several events are important in predicting the retention of odorants in the nose, including air/water partition, diffusion in air, and diffusion in mucus (Hahn *et al*., 1994; Kurtz *et al*., 2004).

Mozell *et al*. (1991) extended the chromatographic model to show that there is an interaction between the sorption properties of odorants and the flow rate in determining the olfactory response. They found that responses to strongly sorbed odorants, benzaldehyde and carvone, grew rapidly with increases in flow rate while responses to poorly sorbed odorants, such as limonene and octane, decreased slightly with increases in flow rate. The results were interpreted as variations in the amount of odorant removed from the airway upstream from the recorded receptors. Benzaldehyde and carvone are sorbed so effectively onto the wall of the nasal cavity that relatively few molecules reach the receptors at low flow rates, but with faster airflow, the upstream sorption is sufficiently decreased to allow a higher concentration to reach the receptors. A similar explanation was proposed for the decrease in response to limonene or octane at increased flow rates. The faster flow was proposed to diminish local entry of the odorant into the mucus layer between the airstream and the cilia olfactory sensory neurons (OSNs). A similar result was obtained by Kent *et al*. (1996) using voltage-sensitive dye recordings from rat olfactory epithelium in a situation where airflow was constrained by a transparent plastic sheet to a position just over the epithelium. They found that carvone responses increased with flow rate while responses to propyl acetate, an odorant found to be weakly sorbed, decreased with flow rate.

Topography of response and its relation to receptor distribution

Early indications of spatial localization of odor responses in the olfactory epithelium came from the works of Daval *et al*. (1970) and Mustaparta (1971), who used the electroolfactogram (EOG) to map responses to direct application of odorants to the frog olfactory epithelium. The EOG is a surface-negative response of the epithelium described by Ottoson (1956) as a summated generator potential in olfactory neurons. Moulton (1976) used these data, along with recording in the bulb from localized stimulation in the salamander epithelium (Kauer and Moulton, 1974), to argue that there are both an inherent pattern (selective sensory neurons) and an imposed pattern (chromatographic) contributing to the spatial distribution of responses in the epithelium. Subsequent recordings from salamander (Mackay-Sim *et al*., 1982; Mackay-Sim and Shaman, 1984) and rat epithelium (Thommesen and Døving, 1977; Mackay-Sim and Kesteven, 1994) supported this concept, but there was no clear correlation of the response distribution with any physical properties or odorant classifications.

The issue of spatial distribution of response has taken on new meaning since the observation receptor genes have a zonal distribution. OSNs expressing different receptor genes are expressed in different parts of the epithelium. The expression pattern in rodents was initially described as comprising anterior-to-posterior–oriented zones with expression of individual genes widely scattered within one of these zones (Ressler *et al*., 1993; Vassar *et al*., 1993; Sullivan *et al*., 1996). I will use the terminology of Sullivan *et al.* (1996), who numbered these zones 1–4, with zone 1 lying in the dorsal recess of the epithelium and zone 4 encompassing both ventral and lateral regions. The early characterization of discrete zones has been modified to some extent by other publications. Strotmann *et al*. (1994) reported a gene that was expressed in a more restricted pattern. Others have reported that some genes are expressed in patterns parallel to the original zone descriptions, but with overlapping boundaries (Iwema *et al*., 2004; Miyamichi *et al*., 2005). The pattern of olfactory gene expression has recently been described for the tiger salamander (Marchand *et al*., 2004) where each of the receptors studied so far is also found in only a part of the epithelium. The expression zones for the salamander also seem to lack sharp boundaries. All these reports agree that OSNs expressing each of the genes are distributed widely within each expression zone.

Our EOG observations (Scott *et al*., 1996, 1997, 2000; Scott and Brierley 1999) from the rat show a general alignment with the expression zones. Our data were obtained with simultaneous recordings from four to eight electrodes placed to sample responses from different expression zones. Odorants were applied by an air puff from a glass tube centered over the surface to be recorded. Peak response voltages were standardized to the response to an isoamyl acetate stimulus to adjust for variations in tissue condition. Photographic overlays of recording sites were used to combine data from multiple animals.

Figure 1 shows the arrangement for simultaneous EOG recordings from Scott and Brierley (1999). These are illustrated on a drawing of the zonal receptor expression pattern based on the data of Ressler *et al*. (1993) and Vassar *et al*. (1993). We conducted these recordings on the epithelium overlying the midline of the endoturbinate bones where we could place eight electrodes, usually in a linear array as illustrated for the sites on the rostral border of endoturbinate IV in Figure 1A. The normalized peak responses were plotted as a function of the linear distance measured along the rostral edge of the turbinate bones. For many of the stimuli the response plots were nearly linear as illustrated in Figure 1B.

For a later report (Scott *et al*., 2000) we sought a way to summarize responses to a larger number of odorants. We used multiple regression to fit linear and second-order equations to those plots and displayed the results in a summary plot showing positive slopes for saturated hydrocarbons that primarily activated ventral sites and negative slopes for

highly polar compounds that primarily activated dorsal sites. The linear slopes from this paper are replotted here in Figure 2 to show the relationship of the linear slope with the Hansen solubility parameter (Burke, 1984), representing the amount of energy required to separate the molecules. Liquids with large-solubility parameters are more easily soluble in water, which has a solubility parameter of about 48. The values were computed with Molecular Modeling Pro v.3.2 software (ChemSW Inc., Fairfield, CA). Figure 2 is plotted with smaller values of the solubility parameter to the right in order to have odorants that evoked larger responses on the ventral part of endoturbinate IV appear on the right side of the plot as in our previous figures (Scott *et al.*, 2000). Other calculations related to solubility, such as log(*P*) or the water solubility in milligrams per liter or moles per liter, also correlate significantly with the EOG response slope, but none gave as good a fit. These data indicate a relationship between the distribution of response to odorants from this sample and some chemical property related to polarity or solubility.

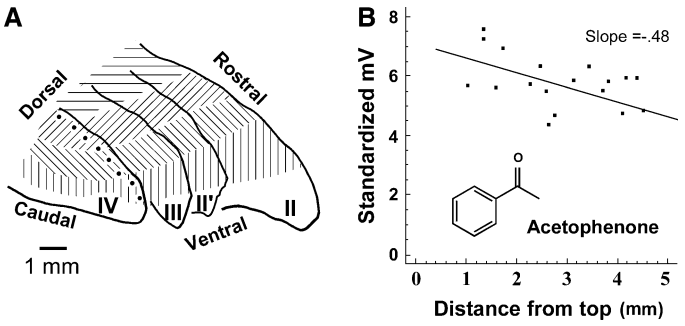

Figure 1 **(A)** A set of eight typical electrode placements along the rostral edge of endoturbinate IV (adapted from Scott and Brierley, 1999). This view is from the midline with the septum removed. The four gene expression zones are adapted from the illustration of the distribution in the neonatal rat (Vassar *et al.*, 1993). **(B)** The distribution of peak responses to the acetophenone odor standardized to the isoamyl acetate response at each site. The linear regression slopes from this type of plot were used to construct Figure 2.

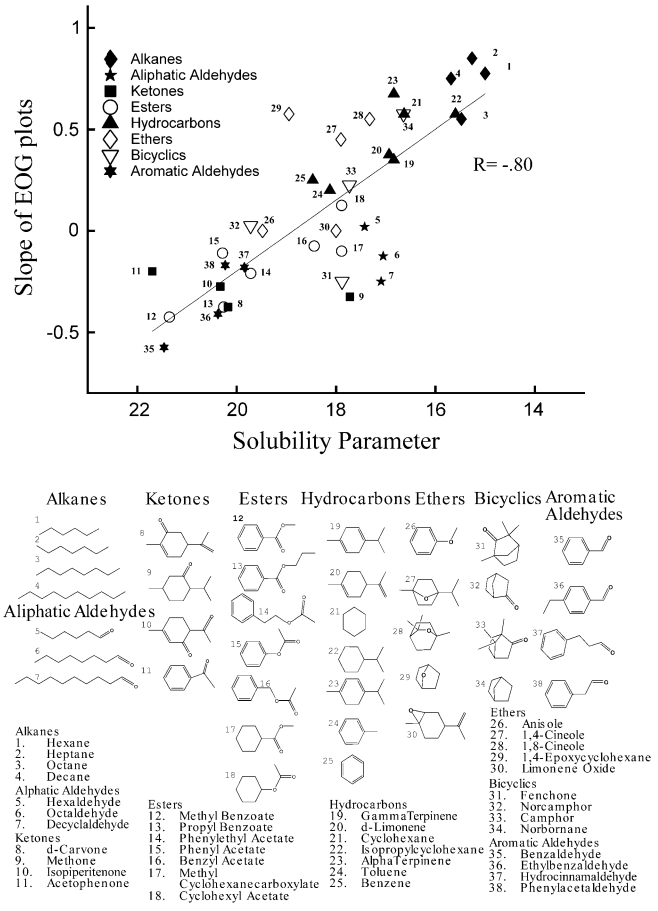

Figure 2 The linear slopes of EOG plots (see Figure 1) graphed against the Hansen solubility parameter. The data are for 38 odorants including acetophenone from Figure 1 and most odorants from Scott *et al.* (2000).

The important cautions about this plot are that it represents only a small subset of possible odorants and that it is based on only the linear part of the graph of response sizes. For some odorants, especially 1,8-cineole, the graph was significantly nonlinear with a peak response corresponding approximately to zone 3 of Figure 1. This nonlinear portion was ignored in the construction of Figure 2. For the most part, the concentrations used for this figure gave responses of approximately equal size. One of the outliers on the plot was the ether 1,4-epoxycyclohexane, which evoked a much larger response than most odorants of the sample. Another set of significant outliers was that of aliphatic aldehydes, suggesting that as a group they may not correspond with the general rule. Nevertheless, the figure shows a continuous relationship across the epithelium in which poorly soluble odorants tend to evoke larger responses in the ventral epithelium.

The retention time on a Carbowax column was useful to Mozell (1966) in predicting the effect of flow rate on responses in the frog nose. Mozell and Jagodowicz (1973) found a strong correlation between retention times on such a column and the retention times in a frog nose when they substituted the frog for the column in a gas chromatography. They also found a strong correlation between the mucosa retention time and the ratio of response in anterior/posterior frog epithelium under normal flow conditions. We were able to get retention time information from the literature (Fuller *et al.*, 1964; Anker *et al.*, 1990; Egolf and Jurs, 1993) for 17 of these odorants. These values also correlate strongly with the EOG slope values in the same range as the solubility parameter for this more limited data set (r = 0.73–0.75, $P < 0.01$).

We tested the pattern of EOG response with a reduced set of stimuli on several surfaces of the exposed epithelium to test the generality of the correlation with gene expression zones (Scott *et al.*, 1996; Scott and Brierley, 1999). The variation in the response profiles along the anterior-to-posterior axis of the zones along the surfaces was small and unsystematic compared to the systematic variation across zones. The contours of polar versus nonpolar responses also followed

the curvature of zonal expression on endoturbinate IV described by Vassar *et al*. (1993) for the rat (though less evident in the description for the mouse by Resseler *et al*., 1993). We also found a polar to nonpolar gradient agreeing with the zonal distribution on the surface of each of the turbinate bones pictured in Figure 1, as well as on the septum and on the dorsal surface of endoturbinate II. Because the properties of the ventral and lateral parts of zones 3 and 4 appear very similar, I will often refer to these portions of the epithelium as ventrolateral in this review.

There are some data in the literature that do not agree with our observations. The EOG recordings of Mackay-Sim and Kesteven (1994) failed to find a zonal arrangement of response. This paper differed from our approach in the odorant sample but also in the use of a single electrode to map each experimental animal rather than the multiple electrode approach that we used. This may have introduced substantial variance. Kent *et al*. (2003) point out that their voltage-sensitive dye recordings do not show a strict zonal localization of odorant response. In some of their papers (Kent *et al*., 1995; Youngentob and Kent, 1995) they describe the distribution as more "hot spots" than zones. It is certainly true that the voltage-sensitive dye technique provides a more extensive spatial sample of the EOG. In fact, our choice of using EOG was clearly a compromise chosen partly because it was available and partly because it allowed direct comparisons with data collected from intact animals and from the exposed surfaces of the turbinate bones. One important difference in procedure is that our odorant sample was chosen to particularly reflect the polar to nonpolar dimension after we noticed that the odorants in our initial sample that produced the most consistently different responses were limonene and carvone, different by only the presence of a carbonyl group. The odorant sample used by Kent *et al*. (1996, 2003) is not as extreme in its chemical properties (e.g., there were no hydrocarbons) and may be more appropriate to showing more subtle variations in the epithelium.

The contrast of the EOG and voltage-sensitive dye recordings is potentially important because the resolution of the EOG is rather poor. The estimates of the EOG space constant from Mackay-Sim and Kesteven (1994) and from Daval *et al*. (1970) are on the order of 100 μm. In addition, the OSNs expressing any particular receptor are not necessarily uniform within an expression zone. Calcium-sensitive dyes tested at a higher resolution to visualize single cells (Sato *et al*., 1994; Ma and Shepherd, 2000) show that OSNs with different response patterns are intermixed within a field of the epithelium. Therefore, one must be careful to regard the EOG distributions as average data. Hamana *et al*. (2003) have reported a series of cells responsive to carvone tested with a set of 21 other odorants and found only a few with robust responses to other odorants. Unfortunately for current purposes, they do not report the zonal origin of these cells. Perhaps future recordings with calcium- or voltage-sensitive dyes will answer the question of whether the greater EOG responses in certain areas (e.g., to nonpolar compounds in ventrolateral regions) represent large responses in a comparatively small number of cells, common properties of all OSNs in the region, or some other alternative.

Recently, Norlin *et al*. (2005) have reported a study with c-fos activation in the mouse olfactory epithelium with a series of odorants. They found labeling of cell populations frequently restricted to one zone but scattered throughout the zone as would be expected from the receptor expression distributions. Their results with benzene and benzaldehyde agree with our localizations of maximal responses, and the strong restriction of responses to single zones may reflect the airflow effects discussed subsequently. Their results with pyridine and pyrazine show zone 4 responses. This is not in agreement with the plot of Figure 2 because these compounds have a high-solubility parameter that would predict a large zone 1 response. This discrepancy could arise because Figure 2 does not contain any nitrogen compounds. The odorant concentrations that these authors used labeled about 1% of cells or less, so they may have selected for the most responsive cells in the epithelium.

One experiment has tested the functional consequences of the zonal distribution behaviorally. Vedin *et al*. (2004) exposed mice to low concentrations of dichlobenil that led to selective damage to zone 1 of the epithelium. They tested the ability to detect a series of odorants (benzene, ethyl acetate, decanol, and pyridazine). This treatment has no effect on the detection of benzene and ethyl acetate (compounds with calculated solubility parameters of 18.5 and 18.7, respectively) but substantially raised the thresholds for response to *n*-decyl alcohol and pyridazine (compounds with calculated solubility parameters of 20.2 and 25.2, respectively). These results are consistent with these odorants having their greatest response in zone 1 and with the conclusion of Figure 2 that the chemical properties of odorants are correlated with the region of maximal response. Unfortunately, this has not been directly tested for either *n*-decyl alcohol or pyridazine.

It is possible that the greatest difference in the epithelium is that between zone 1 and the other ventrolateral regions. Zhang and Firestein (2002) have pointed to the pattern of expression of class I (or "fishlike") receptor sequences which are found only in zone 1 of the rodent. In contrast, the class II sequences are found throughout the epithelium. Miyamichi *et al*. (2005) have also supported this concept. Zhang and Firestein (2002) reviewed data suggesting that the class I receptors may be specialized for water-soluble compounds. Unfortunately, the overlap in odorants tested in the samples they reviewed and those tested for distribution of responses in the epithelium and bulb is not great enough to strongly support a gene structure substrate for the response distributions. The answer to whether there is a predictable gene structure associated with the epithelial response distribution will have to await more data on individual receptor genes correlated with receptor responses.

Finally, in considering the epithelial chemotopy, one should acknowledge that we have no direct evidence that it is produced by properties of the receptor proteins. It is logically possible that some differential access to the receptors exists even in the exposed preparation that we have described. A number of different genes and proteins are distributed in a fashion that parallels the receptor zones (Fülle *et al*., 1995; Norlin and Berghard, 2001; Norlin *et al*., 2001; Whitby-Logan *et al*., 2004; Yu *et al*., 2005). Perhaps some of these influence access of odorants or their degradation. There are now several different identified odorant-binding proteins with different odorant specificities in the rodent (Lobel *et al*., 2002). Several groups have proposed evidence to suggest that multiple genes may be functionally expressed in at least some neurons (Rawson *et al*., 2000; Schild and Manzini, 2004) or that other mechanisms such as inhibition (Sanhueza *et al*., 2000; Delay and Restrepo, 2004; Castillo *et al*., 2005) may partly shape the response. Another potential influence is the potential necessity of chaperone proteins for functional activity in OSNs (Hague *et al*., 2004; Larsson *et al*., 2004; Saito *et al*., 2004) that may determine whether a receptor is actually functional in a particular region of the epithelium. As yet there is no indication that odorant-binding proteins, factors related to inhibition, transmitter receptors, or chaperone proteins are zonal distributed. At present, there does not seem to be a clear alternative to receptor distributions as the mechanism for differential response.

Relation of epithelial chemotopy and olfactory bulb chemotopy

The study of responses of olfactory glomeruli to odorants (reviewed in Nagao *et al*., 2002; Leon and Johnson, 2003) has come to a similar conclusion that in general the response to polar odorants is greatest in glomeruli of the dorsal bulb and the response to hydrocarbons is greatest in glomeruli of the ventral bulb. This arrangement is in concert with the anatomical observations of a diffuse topography in the projections of OSNs to the olfactory bulb described with anatomical tracer techniques (Land, 1973; Astic *et al*., 1987; Stewart and Pedersen, 1987; Schoenfeld *et al*., 1994; Miyamichi *et al*., 2005). The projections of individual OSNs within each zone converge onto a small number of glomeruli, often on one glomerulus for each side of the bulb (Ressler *et al*., 1994; Vassar *et al*., 1994; Mombaerts *et al*., 1996). The observations of Miyamichi *et al*. (2005) suggest that the anatomical projection pattern is not strictly zonal, that is, retrograde labeling from spatially graded sites on the olfactory bulb produces a spatially graded series of labeled OSNs in the epithelium that are not confined to the four zones proposed by Ressler *et al*. (1993). This certainly does not mean that the distribution of response at the glomerular layer accurately reflects the epithelial response distribution. Igarashi and Mori (2005) illustrate several ventral glomeruli with responses to benzaldehyde, carvone, and acetophenone, stimuli that maximally activate the dorsal epithelium in our recordings. Such ventral responses were not so obvious in the Johnson and Leon data (Johnson *et al*., 2002). Some of the ventral glomeruli responding to polar compounds in the Igarashi and Mori (2005) study had broad-spectrum responses, but the authors concluded that in general the responses of ventral glomeruli were determined more by the hydrocarbon structure than by the functional group. If that is the case it may make functional sense that some of the ventral receptors may respond to polar compounds because of their particular hydrocarbon structures. This interpretation would suggest that EOG responses to polar compounds in ventral epithelium arise from particular subpopulations of ORNs. While the imaging of glomerular response tends to support our conclusions from the EOG recordings, they also suggest a diversity of response in the various parts of the epithelium that is consistent with the epithelial imaging studies that gave higher resolution.

Responses in the olfactory bulb clearly reflect properties that are not evident in the epithelial recordings. This is most evident for the comparison of carbon chain length, for which variations produce systematic changes in the position of maximal response in the bulb (Rubin and Katz, 1999; Johnson and Leon, 2000; Xu *et al*., 2003; Takahashi *et al*., 2004). Our EOG recordings do not show these properties (Scott *et al*., 1996). On the other hand, Kent *et al*. (2003) were able to use voltage-sensitive dye data from the epithelium to predict the behavior of rats in a confusion matrix task. It is not possible to conclude from that experiment that any feature other than the zonal distribution was responsible for that prediction. The mechanism by which these more precise maps of odorant properties arise in the bulb in development is a matter of active investigation (Tsuboi *et al*., 1999), but it is important to note the possibility that intrabulbar processing may affect some of the properties seen with some of these measurements (Wachowiak and Cohen, 2003).

Interaction of flow rate and receptor topography

The nasal cavity has some regions of large cross-sectional bore and other regions of smaller cross bore. These would appear to lead to different flow rates in the nose. Indeed a number of models (Kimbell *et al*., 1997; Zhao *et al*., 2005) have provided support for this idea. Ezeh *et al*. (1995) and Scott-Johnson *et al*. (2000) reported differences in the latency of EOGs recorded from the dorsomedial and ventrolateral recesses of the epithelium, which are likely to have been generated by the differential flow rates. Figure 3 shows recordings from an intact rat with airflow drawn through the nose in an artificial sniff at varying flow rates. The responses in the dorsomedial recess consistently begin earlier and the difference between the dorsomedial and ventrolateral sites is greater at low flow rates. This is typical of the delays that we observe in this preparation. These rates overlap the range that Youngentob *et al*. (1987) reported

for sniffing in behaving rats. The lowest average inspiratory flow that he observed was about 180 ml/min when the animal was sniffing at a strong odor. It is quite possible that quiet breathing may involve lower flow rates. They found conditions where the maximum inspiratory flow could exceed a liter per minute. We rarely have tested such high rates, but Figure 3 predicts that there would be minimal delay between the two sites at such high flow rates.

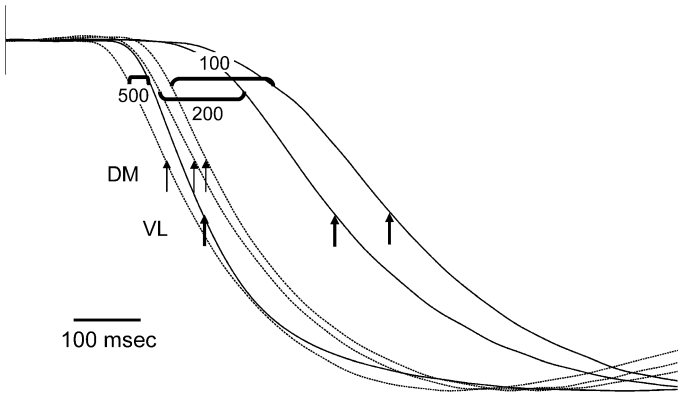

Figure 3 An example from a single animal of simultaneous EOG recordings from the dorsomedial recess (DM) and ventrolateral (VL) epithelium in an intact nose with an isoamyl acetate odor at a nominal dilution of 10^{-2}. The traces show the mean of three responses. Three flow rates were used: 100, 200, and 500 ml/min with a sniff duration of 2.5 s. The responses are normalized to the peak voltage for this figure to allow comparison of the time course. The dotted traces with thin arrows are the recordings' positions from the dorsomedial recess. The solid traces with thicker arrows are the recordings from the ventrolateral epithelium. The brackets connect records recorded at the same flow rate. Note that the delays in the ventrolateral epithelium are much greater at the lower flow rates.

The effect of flow rate on odor response reported in the frog nose (Mozell *et al*., 1991) suggested that the geometry and flow rates in the rat nose would have significant effects on responses to odorants with different physical and chemical properties. Because of the complex geometry of the rodent nose, it was not possible to reverse flow as was done for the frog (Mozell *et al*., 1987). However, Scott-Johnson *et al*. (2000) tested this with recordings that compared an exposed epithelial preparation like that illustrated in Figure 1 with an intact preparation like that in Figure 3. The results are summarized in Figure 4, which compares the response in the exposed preparation to responses of an intact nose at a 500-ml/min flow rate for a 2.4-s stimulus. This very long stimulus allowed all EOG responses to reach a maximum. We found that carvone and benzaldehyde, highly polar odorants that should be readily sorbed onto the epithelium, gave smaller responses in all parts of the epithelium of intact animals than on the exposed epithelium. This is consistent with their being removed from the airstream before reaching the receptors. This effect was particularly dramatic for the ventrolateral epithelium. In the exposed epithelium the benzaldehyde ventrolateral response was approximately half the size of the dorsomedial response, but in the intact animal the ventrolateral response was almost undetectable. For the poorly sorbed odorants, benzene and limonene, the responses on the ventrolateral exposed epithelium were about 20% larger than the dorsomedial responses. In contrast to the other compounds, the ventrolateral responses to these odorants

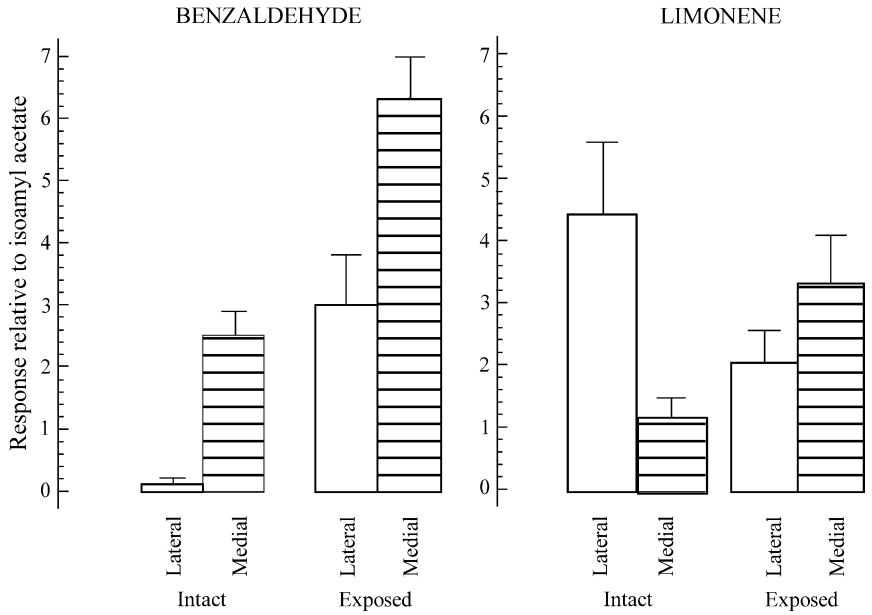

Figure 4 The comparison of responses to a polar odorant (benzaldehyde, 35 in Figure 2) and a relatively nonpolar odorant (limonene, 20 in Figure 2) in the intact and exposed epithelium redrawn from Scott-Johnson *et al*. (2000). The exposed epithelium points were taken from the most medial and lateral regions of the superior surface of endoturbinate II. In both cases, the odor pulse is of sufficiently long duration to allow the EOG to reach peak amplitude (0.6 s for the exposed preparation and 2.4 s for the intact preparation). The means and standard errors are normalized to the mean isoamyl acetate responses for each site and condition.

in the intact animal were approximately the same size as in the exposed animals, but the dorsomedial responses were greatly reduced. Changes in flow rate or pulse duration also changed the response size, particularly for the more polar odorants. These results indicate that the morphology of the nasal cavity substantially influences the response.

The striking similarity of the airflow pattern in the Kimbell *et al.* (1997) model with the receptor zonal distribution suggests a functional interaction between the flow patterns and receptors. Our data from both the exposed rat epithelia show that there are inherent mechanisms, presumably related to the receptor distribution, that impose greater sensitivity to polar, more hydrophilic compounds in regions where the airflow is high and greater sensitivity to nonpolar, hydrophobic compounds in regions where the airflow is low. The latency data from the intact nose show that the airflow model is correct in projecting a slower transit time for air through the lateral recesses of the epithelium. The response size data from the intact nose versus exposed epithelium show that these flows influence the response. Polar odorants apparently reach the lateral recesses in only small amounts, even when the response is allowed to reach its maximum with an unphysiologically long stimulus pulse.

Recent observations from the salamander support this interaction of airflow and receptors. Marchand *et al.* (2004) have described the patterns of distribution of 20 candidate olfactory genes in the salamander epithelium. These tend to lie in bandlike patterns perpendicular to the pattern of airflow from the external to internal nares. They have drawn a remarkable comparison between the expression patterns and the published spatial response distributions from salamander. They have pointed out that the responses to hydrophilic odorants are greatest close to the inflow at the external nares and the responses to hydrophobic odorants greatest near the internal nares. All these recordings come from exposed salamander olfactory epithelium so there have been no direct comparisons of the intrinsic receptor responses and responses imposed by the airflow in salamander. The difference between the receptor distribution pattern in the geometrically simple amphibian nose and more complex rodent nose is very interesting. It suggests that the evolution of the rodent expression pattern has taken advantage of the greater flow differences possible in a larger, more convoluted nasal cavity. The relatively poor olfactory abilities claimed for the human nose may have something to do with the retreat of our olfactory epithelium to a relatively small patch as well as the smaller numbers of receptor genes, although Zhao *et al.* (2004) have concluded that the human nose probably does sustain very substantial differences in flow rate.

The role of the sniff in temporal processing

These indications that airflow is slower in the ventrolateral epithelium may have implications for temporal processing in the olfactory bulb. The longest delays that we see between the EOGs in dorsomedial and ventrolateral epithelia are long relative to the average duration of about 60 ms for an inspiratory sniff in a rat detecting a low odor concentration (Youngentob *et al.*, 1987) and greatly exceed the duration of the initial burst of spikes often seen in an odor response (Onoda and Mori, 1980; Harrison and Scott, 1986; Wellis *et al.*, 1989; Chalansonnet and Chaput, 1998; Giraudet *et al.*, 2002). These delays cannot be completely compensated for by differential conduction velocity in the axons of OSNs because they change with airflow velocity. One function of the sniff may be to reduce these delays so that all inputs reach the bulb at the same time.

Is it possible that the delays induced by flows in the epithelium could provide a mechanism for encoding temporal response patterns? With low flow rates, the delays would be very long relative to either the period of the gamma or beta oscillations observed in the olfactory bulb (Kashiwadani *et al.*, 1999; Kay, 2003; Neville and Haberly, 2003). Therefore, it does not seem likely that the delays in the epithelium would be a useful mechanism for encoding any phase relationships with these fast oscillations, in part because the delays would change with the sniff velocity. It is not likely that a stereotyped high-velocity sniff could act to standardize these delays to make phase coding possible because the relative patency of the two nostrils is not constant over time (the issue of the nasal cycle is reviewed in Eccles, 2000, and Frye, 1995). However, one possible function of the sniff is to decrease the relative latency of responses in ventral parts of the bulb to bring them into the same temporal domain as dorsal responses. This could enhance the spatiotemporal mechanisms in the bulb.

General conclusions

Several findings from the last decade have greatly increased the understanding of the long-standing question of the roles of airflow and of local odorant sensitivity in the olfactory epithelium of amphibians and rodents. The expression patterns of receptor genes correlate with the airflow patterns in both rats and salamander. They also correlate with the relative sensitivity to hydrophilic and hydrophobic odorants across a series of hydrocarbons and oxygen-containing compounds. This pattern of sensitivity is optimally placed to take advantage of patterns of sorption of odorants out of the airstream. The effect of sniffing at different velocities may change this relationship.

The sniff is clearly important in regulating the amount of odorant entering the nose. There is also reason to believe that the type of odorant reaching the receptors can also be regulated by the intensity of sniffing. Finally, we have asked whether the temporal aspects of the response in the olfactory bulb are modulated by centrifugal inputs or by odorant properties. These are unresolved issues, but it seems likely that the dynamics of the sniff impose important structure on our olfactory experience.

Acknowledgements

I thank Lisa Sherrill and Humberto Acevedo for editorial help and help in producing data and figures. I thank Tom Schoenfeld for suggesting the analysis used in Figure 2. The work from my laboratory described here has been supported over the years by a grant from the National Institute of Deafness and Other Communication Disorders.

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Accepted November 9, 2005