Chem. Senses 31: 581–593, 2006

doi:10.1093/chemse/bjj063
Advance Access publication June 1, 2006

Effects of Concentration and Sniff Flow Rate on the Rat Electroolfactogram

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Abstract

Previous reports using the electroolfactogram (EOG) to study the spatial and temporal aspects of response in the rodent olfactory epithelium had focused on high odorant concentrations that gave large responses. This investigation has used lower concentrations to test the difference between responses in the rat dorsomedial and lateral recesses with a range of nasal flow rates and a range of chemical properties. The responses to a highly polar, more hydrophilic odorant changed more steeply with flow rate than responses to a very nonpolar, hydrophobic odorant. With low flow rates there was a response delay in the lateral recess, which is consistent with the models indicating lower flow rates in that region. We observed significant volume conduction effects in which large responses in the dorsomedial region obscured smaller initial portions of the lateral responses. These effects could be removed by destroying the dorsomedial response with a high concentration of a low molecular weight ester. We caution that investigators of EOG recordings from the intact epithelium must attend to the possible presence of volume conduction, which can be assessed by attention to the selectivity of odorant response, response waveform, and response latency.

Key words: hydrophobicity, olfactory coding, olfactory epithelium

Introduction

Modeling of airflow in the rat nose (Kimbell *et al.*, 1997; Zhao *et al.*, 2006) suggests a remarkable agreement between the flow rates during a sniff and the zonal distribution of olfactory receptors (Ressler *et al.*, 1993; Vassar *et al.*, 1993). In those models, the flow rates are high in the dorsal recess (zone 1 of Ressler *et al.*, 1993) and low in the lateral recesses of the epithelium (which express the receptors of zones 2 through 4). As Schoenfeld and Cleland (2005, 2006) have pointed out, this may be primarily due to a large difference between the dorsal recess and the lateral, and there is little evidence at this point of physiological differences among zones 2 through 4 in the lateral recesses. Recording of electroolfactograms (EOGs) have shown that hydrophilic odorants evoke larger responses in zone 1 while hydrophobic odorants evoke progressively larger responses in zones 2 through 4 (Scott *et al.*, 1997, 2000; Scott and Brierley, 1999). Most of these recordings were done along the midline, rather than in the lateral recesses that contain a large portion of zones 2–4. The same relationships of zone and odorant properties were shown for the lateral recess. Class I olfactory receptors, thought to respond primarily to water-soluble odorants, are primarily found in zone 1 (Zhang and Firestein, 2002; Tsuboi *et al.*, 2006). Electrophysiological (Nagao *et al.*, 2002; Takahashi *et al.*, 2004; Igarashi and Mori, 2005) and metabolic (Johnson *et al.*, 2002; Leon and Johnson, 2003) mapping of the olfactory bulb have reported a corresponding localization of response to hydrophobic and hydrophilic odorants in the olfactory bulb. Although there is a generalized topography in the projection from the olfactory epithelium to the olfactory bulb (Astic *et al.*, 1987; Schoenfeld *et al.*, 1994; Miyamichi *et al.*, 2005), it is quite clear that the responses in the bulb do not represent a point-to-point projection from the epithelium. In fact, for all receptors studied by *in situ* hybridization, the labeling is distributed in longitudinal bands throughout a large area of the epithelium (Ressler *et al.*, 1993; Vassar *et al.*, 1993; Mombaerts *et al.*, 1996). Both Iwema *et al.* (2004) and Miyamichi *et al.* (2005) have indicated that instead of discrete zones there may be a continuum of areas in the lateral epithelium expressing different genes and projecting to glomerular sites with systematically different dorsal-to-ventral positions. The general agreement of a strong convergence of input to the olfactory glomeruli highlights the question of whether there is a functional consequence of the zonal (or near zonal) distribution of receptors in the epithelium.

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We have suggested that there has been a coevolution of the expression patterns in the epithelium with the airflow patterns so that receptors sensitive to hydrophobic or hydrophilic odorants are placed where they would encounter the highest concentration of the appropriate stimulus (Scott and Scott-Johnson, 2002; Scott, 2006). Marchand *et al.* (2004) made a similar suggestion in pointing out the correlation of odorant receptor gene expression patterns in the salamander olfactory epithelium with the spatial pattern of multiunit responses to odorants. A similar view has been expressed in a recent review by Schoenfeld and Cleland (2006). These proposals are originally based on the observations of Mozell *et al.* (1991) and Kent *et al.* (1996) who demonstrated in the frog nose that high flow rates favor the response to hydrophilic odorants, and low flow rates favor response to hydrophobic odorants. They reasoned that high flow rates minimize the sorption of odorants onto the walls of the nasal cavity upstream of the receptors. Similarly, low flow rates maximize the sorption of odorant into the mucosa allowing it to reach the receptors. Their interpretation is founded in earlier experiments showing that the gas chromatographic retention time of odorants on a polar column is related to the gradient of odor response across the epithelium (Mozell and Jagodowicz, 1973) and to the uptake of radiolabeled odorants into the epithelium (Hornung and Mozell, 1977).

It has not been possible to make direct measurements of airflow in the intact rodent nose, but previous reports from our laboratory (Ezeh *et al.*, 1995; Scott-Johnson *et al.*, 2000) have indicated a delay in response for the lateral recesses compared to the dorsomedial recess. Such delays are consistent with the airflow models (Kimbell *et al.*, 1997; Zhao *et al.*, 2006). The convergence from all along the length of the zone to a single glomerulus (Mombaerts *et al.*, 1996) suggests that the spatial pattern of sorption along that length may not be the important issue. More likely, the important factor is that low versus high flow rates differentially favor sorption of certain odorants depending on their solubility (Mozell *et al.*, 1991). In this paper, we explore the effects of odorant solubility, concentration, and nasal flow rate on responses in the dorsal and lateral recesses.

The EOG is a valuable technique for observing the dynamics of response in the epithelium. Because it is a population response, it is not biased to the response of an olfactory receptor neuron (ORN) expressing one of the 200 or more receptor genes that are expressed in a particular zone (or zonal strip). However, the EOG does have some limitations that require exploration. One of these issues is the possibility that some EOG waveforms might be contaminated by current paths from distant active sites (Scott and Scott-Johnson, 2002). It seems appropriate to apply that concern to our own reports (Ezeh *et al.*, 1995; Scott-Johnson *et al.*, 2000) where signals of several millivolts generated in one part of the epithelium may affect the recording of smaller potentials in another part of the epithelium. This is a more complicated issue than the estimate of the space constant of the epithelium at about 100 μm in flat regions of the rat (Mackay-Sim and Kesteven, 1994) or frog (Daval *et al.*, 1970) epithelium because of the highly folded nature of the lateral recesses of the rat epithelium.

We have observed considerable variation in the waveforms and latencies of EOGs recorded from the lateral recess of the epithelium of intact rats. These variations were prominent when lateral responses were small. In the past, we dealt with this problem by excluding data from animals with small responses; however, we wished to evaluate the latency question more closely. We argue in this report that the lateral waveforms are influenced by volume conduction from the shorter latency dorsomedial responses, but this problem can be greatly reduced by attention to the form and latencies of the responses with low nasal flow rates. We have tested this model by intense stimulation with a short-chain ester (methyl propionate) that abolished the dorsomedial responses. This allowed more accurate measurement of waveforms and latencies in the lateral recess. These measurements have supported the prediction of lower flow rates across this part of the epithelium.

Materials and methods

Surgery

Male Sprague–Dawley rats (225–375 g) were rapidly killed by overdose with sodium pentobarbital (200 mg/kg). This reduced bleeding and maintained maximal patency of the airway. For study of sniff flow rate, we used rats with an intact nasal cavity as described previously (Scott-Johnson *et al.*, 2000; Ezeh *et al.*, 1995). For this preparation, odors were pulled into the nose with vacuum applied at a cannula inserted through the trachea and resting above the soft palate. The bone overlying the dorsomedial recess of the nasal cavity was thinned with a dental burr until it could be picked off gently without damage to the epithelium. A similar exposure was made to expose ventrolateral olfactory epithelium between the bases of endoturbinate II and ectoturbinate II. Particular care is taken to avoid removing the lacrimal bone or tearing the soft tissue at the rostral part of the orbit, which would allow a leak into the airway and prevent adequate olfactory stimulation.

For direct odor application to the exposed olfactory epithelium, we used the approaches described by Scott and Brierley (1999) to expose the dorsal surface of endoturbinate II and the medial surfaces of endoturbinate IV. For this experiment, we changed our previous procedure to flow mammalian Ringer's over the epithelium at a low rate (~0.1 ml/min). This prevented drying and allowed us to stimulate with lower stimulus concentrations than in the past.

Odor stimulation

Odorants were introduced with an 8-mm glass tube with multiple ports connected to bottles containing test stimuli.

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Odorants were diluted in mineral oil at ratios between 0.1 and 0.001 and kept in glass bottles with gas tight Teflon fittings and tubing leading to the stimulus tube. These dilutions were tested with a gas chromatograph equipped with a flame ionization detector when they were made up, and they were confirmed after several days of use. These bottles had a volume of 100 cc. The mineral oil/odorant mixture covered the bottom of the odor sample bottle to a depth of ~0.5 cm and had a surface area of about 6 cm^2. Air flowed through the odor sample at a rate of 100 ml/min. The sample was further diluted by injection into an air stream that constantly flowed over the preparation at a rate of 1000 ml/min so that the final odorant dilutions ranged from 0.01 to 0.0001 times saturation. Odorant flowed in the system for 9 s to allow the odorant concentration to stabilize in the system before the vacuum was turned on to initiate the 1-s sniff. This provided a temporally sharp odor onset. The stimulus control programs were written in LabView to allow flexibility in control of durations. For experiments comparing sniff flow rates in the nose, three parallel needle valves were used to adjust the flow in lines connected to valves controlled by the program. These were adjusted with a float-type flowmeter to provide nominal flows of 100, 200, and 500 ml/min at steady state. These rates were measured in the line leading to the tracheal cannula. Since there was some resistance (including a filter to block moisture) in that line, we cannot be sure of the instantaneous flow rates.

There were two presentations of each odorant in an ascending sequence of response sizes. Whenever flow rates or concentrations were compared in a stimulus series, they proceeded from low to high to minimize adaptation of receptors. Approximately 60 s elapsed between each stimulus presentation. All series contained a blank stimulus to test for contamination or other artifacts. These were presented after each strong stimulus. In cases with methyl propionate treatment, the entire sequence, including blanks, was presented again after treatment.

In order to provide a temporally sharp odor onset for the exposed epithelium recordings, a short "T" connection was inserted between the odor mixing tube and the preparation. When odorant flow began, a vacuum was applied to this T to pull the odorant away for 9 s. The turning off of this vacuum initiated stimulation. This is a modification of the procedure of Kauer and Moulton (1974) and is the procedure used by Scott and Brierley (1999). For both preparations, there was some delay between the valve closing and odorant reaching the epithelium. For the intact preparation, this resulted from the dead space in the vestibule of the nose. For the direct application preparation, the delay resulted from the dead space in the T joint and the distance from that to the epithelium.

Recordings

Recordings were made with glass micropipettes filled with Ringer's solution and broken to a resistance generally less than 10 MΩ. The ground was a silver wire embedded in EEG electrode paste on the frontal bone. The amplifier frequency band was from 0.1 Hz to 30 Hz. Data were digitized at 1 kHz and were analyzed off-line with routines written in MATLAB by one of the authors (J.W. Scott).

For the intact nasal cavity experiments, a small opening (about 5 mm by 2 mm) was drilled in the nasal bone overlying the dorsomedial recess. The micropipette was inserted through the epithelium until there was a maximal response to a test stimulus (isoamyl acetate diluted 0.01). A chlorided silver ball electrode was placed on the outside surface of the epithelium as close as possible to the micropipette. Similar opening and electrode placements were made in the far lateral recess just above the base of endoturbinate II. Thus, we could compare the recordings in the lumen and at the lamina propria for each site as one measure of passive current contamination. We quickly found that bipolar recordings between the lumen and lamina propria did not solve the volume conduction problem.

For the exposed recordings, four micropipettes were spaced at equal distances in a line across the epithelium of the medial surface of endoturbinate IV, spanning the most dorsal and ventral regions from which we could record large responses. The ventral extent of the olfactory epithelium can often be distinguished visually by a slight change in color and in apparent thickness of the epithelium. The electrodes were advanced from above until the first point of electrical contact. The pattern of response across the four electrodes was used to confirm that we had spanned a region equivalent to the recordings of Scott *et al.* (2000), but we only report the data from the most dorsal and most ventral electrodes as being the closest in properties to those of the intact preparation. We have previously shown similar patterns of response across the dorsal surface of endoturbinate II, which would correspond most closely to the positions in our intact recordings (Scott and Brierley, 1999; Scott-Johnson *et al.*, 2000).

Selective damage of the dorsomedial olfactory epithelium in the intact preparation was produced by multiple presentations of saturated methyl propionate stimulus for 10 s until we saw a substantial decrement of the isoamyl acetate response on the dorsomedial site. An alternative method would have been systemic injection of dichlobenil, shown by Vedin *et al.* (2004), to selectively destroy the dorsomedial region (zone 1) of the epithelium. However, that method would not have allowed the comparison of dorsal responses and lateral responses in the same animal because that procedure appears to take at least several hours to have its effect.

Data analysis

Response peaks for the intact preparation were normalized to the response to isoamyl acetate at a dilution of 0.001 and a flow rate of 500 ml/min. Unnormalized responses to this stimulus were not significantly different for the dorsomedial and ventrolateral recording sites. This normalization

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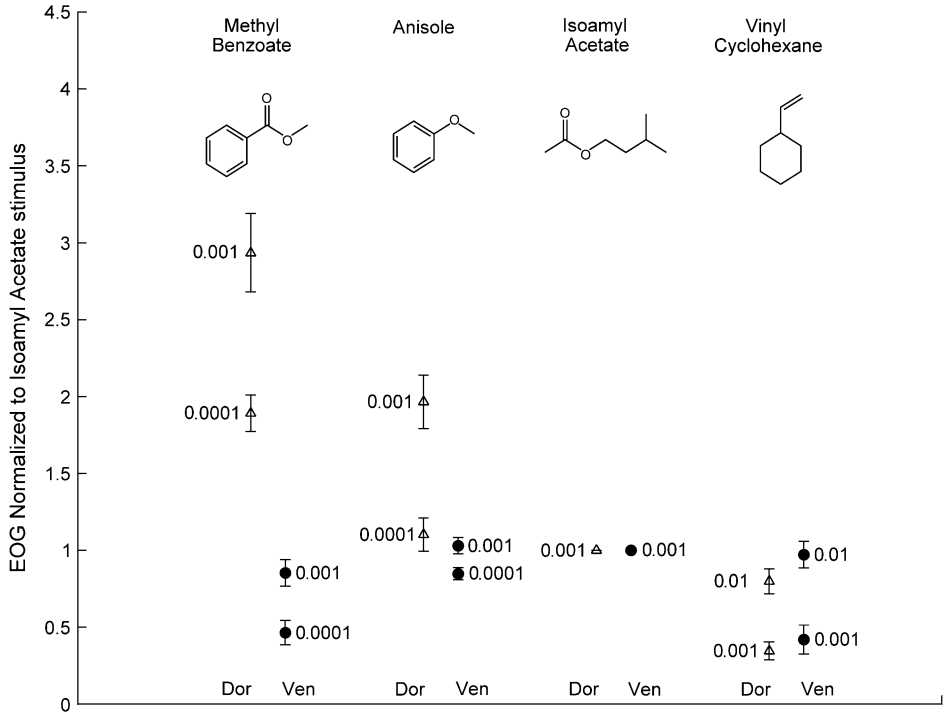

Figure 1 EOG peaks (mean ± SEM, $N = 4$) for dorsal (Dor, open triangles) and ventral (Ven, closed circles) sites in response to four odorants for the opened preparation. In this figure only the electrodes were arrayed dorsal-to-ventral along the surface of endoturbinate IV as in the figures of Scott *et al.* (2000). These values are normalized to the isoamyl acetate response at a dilution of 0.001. The structure of each odorant is shown as insets. The odorants are arrayed left-to-right in order of declining values of the Hansen solubility parameter (21.4, 19.5, 17.1, and 16.3, respectively). The differences across the dorsal responses and across the lateral responses are significant ($P < 0.02$) by the Friedman test, showing that each site response relatively better to some odors than others.

reduced variation from the depth of electrode placement, local damage, or differences in sensitivity between animals. A similar normalization was used in Figure 1 for the recordings from the open nasal cavity experiment. Our previous experiments (Scott and Brierley, 1999) had indicated no systematic difference across the epithelium in response to this stimulus at a higher concentration.

All data were reviewed before analysis. In case of large artifacts or baseline drifts that distorted the response, the distorted record was removed. Otherwise, the two records for each stimulus were averaged for further analysis. In no case was it necessary to remove both records.

Latency measurements were calculated by fitting a template that approximated the initial phase of the response up to 50% of the peak. A computer program matched this template at a series of sizes and latencies to each response with a least squares criterion. The calculated latency was the time of the maximum change in slope of that template. Slopes were calculated as the slope of the linear part of the template up to 60% of the peak. For six of the responses at the lowest flow rate, there was not an adequate fit within the interval from 50–600 ms after odor onset, and these were treated as missing values for calculations of latency and slope. Slopes were expressed as percent of peak response per millisecond. The R^2 for the regression of the response for the 10–60% values with the linear slope was usually greater than 0.99. It was less than 0.8 in only three instances, which were all excluded from the analysis of slopes. This procedure is illustrated in Figure 6 of the results section.

Statistical analysis was from the statistical package of MATLAB. The variances in peak EOG responses to different concentrations and flow rates were not homogeneous. Therefore, statistical tests for multiple comparisons were performed with the Wilcoxon rank sums test (Mann–Whitney *U*-test) or the Friedman two-way analysis of variance in the comparison of EOG peak sizes, where there were no missing values. Tests of overall significance for comparison across odorants, flow rates, or concentration were made after calculating the mean response across the other two variables to avoid inflating the sample size. There were missing values for the measurements of latency and slope for some of the methyl benzoate and anisole stimuli at low concentrations and/or low flow rates. In these cases, the tests were run on smaller numbers of animals and excluding the low concentration. All such exceptions are identified in the figure captions.

Results

EOG magnitudes

Responses from the exposed epithelium follow the pattern described previously (Scott *et al.*, 1997, 2000; Scott and

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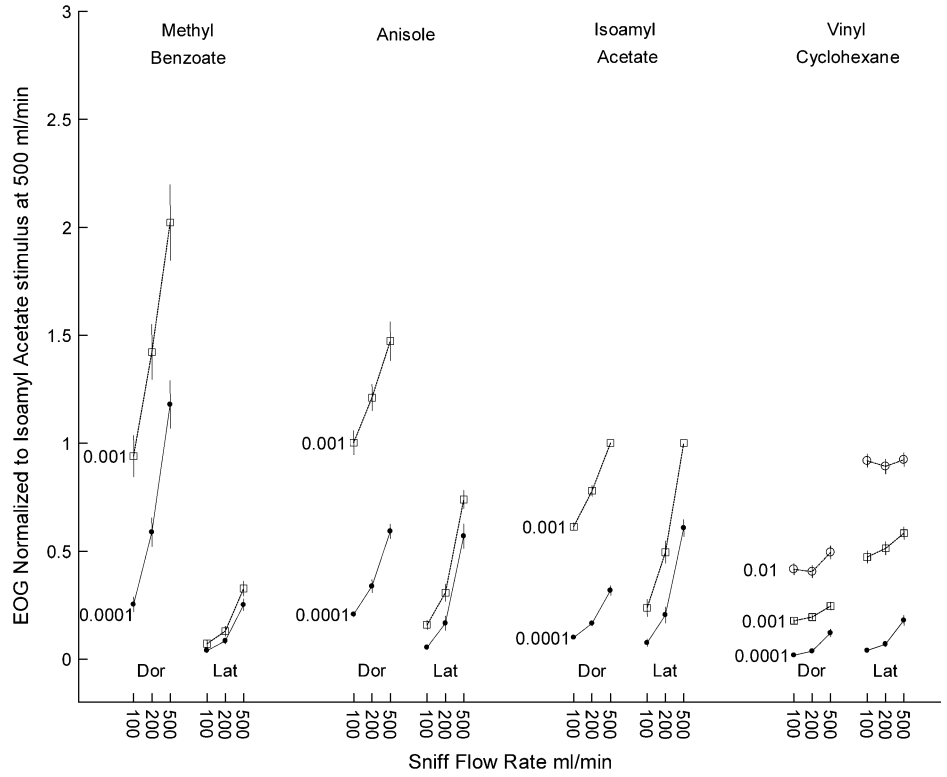

Figure 2 EOG peaks (mean ± SEM, $N = 15$) for dorsal (Dor) and lateral (Lat) sites in response to four odorants at two or three concentrations. As in Figure 1, all responses are normalized to the isoamyl acetate response at the highest flow rate (500 ml/min) and highest concentration (0.001 times saturation) for each recording site in each animal. For each odorant, dorsal responses are represented at the left and lateral responses at the right.

Brierley, 1999). The stimuli used here are well below the concentrations used by Scott and Brierley (1999), which ranged from 0.003 to 0.1 times saturation. Data in this experiment were also normalized to a lower concentration of isoamyl acetate. Figure 1 shows average normalized responses from four animals for the extreme dorsal and ventral sites along the medial surface of endoturbinate IV. The other two electrodes had responses of intermediate size for these odorants. The sizes of response peaks overlap for the ventral site while the dorsomedial responses are much larger for anisole and methyl benzoate, respectively. The size of dorsomedial response peaks is in the order of the Hansen solubility parameter. The Hansen solubility parameter (Burke, 1984) represents the amount of energy required to separate molecules of a compound. Liquids that are highly soluble in water have high solubility parameters. The difference in the dorsal minus the ventral response across odors is significant by the Friedman test ($P < 0.02$). A similar order of effectiveness was seen when responses to the same four odors were recorded across the dorsal surface of endoturbinate II in the extreme dorsomedial versus lateral positions (data not shown). Except for the lower concentrations, these results mostly repeat our previous results at lower concentrations, so a more extensive series was not undertaken.

These four odorants were tested for the intact epithelium at a series of concentrations and nasal airflow rates are shown for 15 animals in Figure 2. The distribution of responses at the high flow rates is similar to that seen for the open preparation in Figure 1. This indicates that the combination of high flow rates and long duration stimuli saturate the upstream sorption of the nasal cavity that control access to ORNs. This sorption does not appear to be saturated for the lower flow rates, except for the most hydrophobic odorant, vinyl cyclohexane. The overall differences between the dorsal versus lateral peak responses across the four odorants are significant by the Friedman test ($P < 0.001$) at each flow rate and for both high and low concentrations. The difference between the dorsal and lateral responses is greater for the 500 ml/min rate than for the 200 ml/min rate and is greater for the 200 ml/min rate than for the 100 ml/min rate for each set of high or low concentrations ($P < 0.001$ by the Friedman test in each case). The relationship between flow rate and response was explored by comparing the slopes of this plot for each odorant. For the dorsal site, slopes were in the order methyl benzoate > anisole > isoamyl acetate > vinyl cyclohexane. When averaged across concentration, the slopes are significant ($P < 0.001$ by the Friedman test). They are similarly significant for either the low or high concentrations. In multiple comparisons, the slope for methyl benzoate is significantly greater than the slope for vinyl cyclohexane ($P < 0.01$ by the Scheffé procedure). There are also overall differences in slopes for the lateral responses, but the

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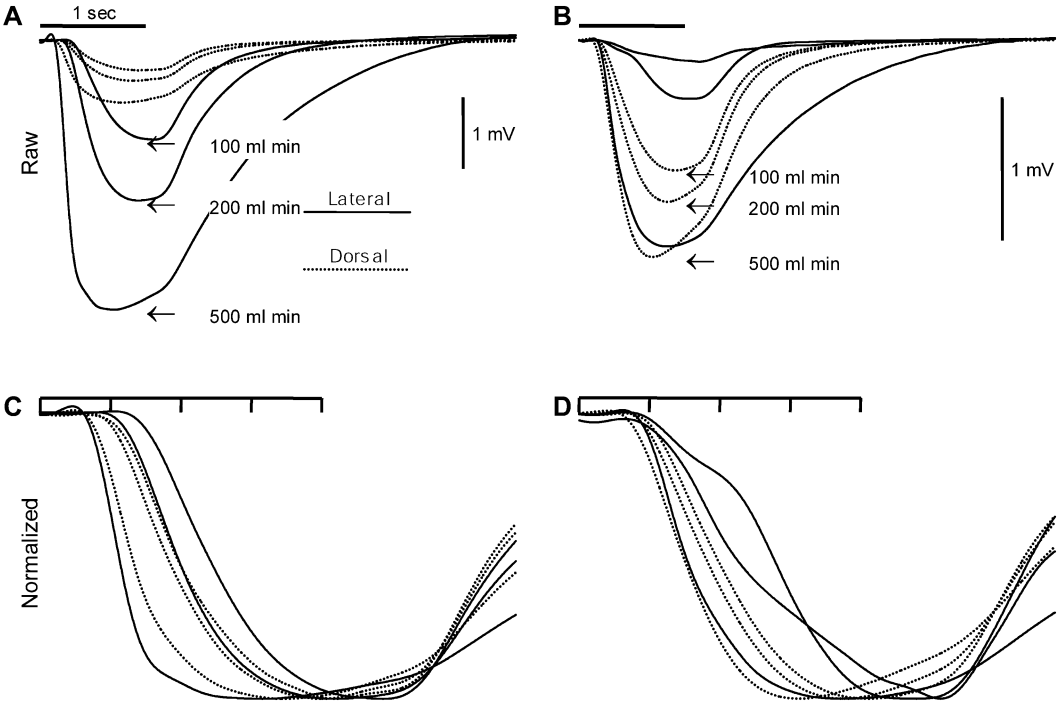

Figure 3 Examples of simultaneous dorsal and lateral recordings from intact rats illustrating EOG waveform problems. This shows a response to isoamyl acetate at dilutions of 0.001 times saturation at three sniff flow rates for two rats. The stimulus duration is 1 s corresponding to the time bar at the top of each set of traces. Each trace is the average of two responses. Panels **(A)** and **(B)** show raw data with the dorsal site represented in dotted lines and the lateral site represented as solid lines. Panels **(C)** and **(D)** show the same responses normalized to the peak voltage and at an expanded time base. The 1-s time bar for the lower panels is marked with 0.250 ms tics. In all these traces, the fastest onsets were to the highest flow rate, and the slowest onsets were to the lowest flow rate. The responses were successively larger and faster as flow rate increased. The records for the animal in (A) and (C) show a distinct delay for the two lower flow rates. These delays were greater for the lateral responses, except at the highest flow rate. The records in (B) and (D) have multiple inflections, and the lateral responses all appear to begin at a similar time point. These waveforms made latency measurements extremely difficult.

order of the slopes is different. The only significant individual comparisons are between the isoamyl acetate and vinyl cyclohexane curves ($P < 0.01$ by the Scheffé procedure), which are significant for the mean values as well as at both concentration ranges.

EOG latencies and slopes

We wished to evaluate EOG latencies and shapes as evidence about the mechanisms that govern the differences in response at the dorsomedial and lateral sites in Figure 2. However, we found substantial inconsistency in apparent time difference between the dorsomedial and lateral responses, which made interpretation difficult. Figure 3 compares responses to isoamyl acetate at 0.001 times saturation in two rats. The recordings in part A showed longer delay for the lateral response at slower flow rates and a relatively simple waveform. In contrast, the lateral responses in part B showed inflections that are not present in most reports of EOG recorded with direct application of odorants to the open epithelium (Scott and Brierley, 1999; Zhao *et al.*, 1998; Wong *et al.*, 2000). These waveforms in the lateral responses made calculation of latency or response rise time difficult. In some rare cases, both the waveforms and the order of effectiveness of odorants were the same on both sites. We suspected that the nearly identical responses in these cases were the result of volume conduction of the dorsomedial response into the lateral space, which is very narrow and where it is difficult to accurately place electrodes. Recordings from silver ball electrodes placed on the outside surface of the lateral epithelium sometimes showed waveforms substantially like those of the dorsomedial epithelium (data not shown) further supporting this interpretation.

We sought a criterion for testing the interpretation of volume conduction. Differential recordings between the surface and luminal electrodes did not reliably remove these complex waveforms. We assume that this is due to the very complex current paths within the complicated turbinate spaces and to the difficulty of placing the silver ball indifferent electrodes close enough to the micropipette without breaking it. Therefore, we took the approach of destroying the response in the dorsomedial epithelium to analyze waveform of the remaining lateral response. We found that long-duration (2.5 s) stimulation with saturated methyl propionate vapor at a low flow rate (50 ml/min) abolished dorsomedial responses within a few trials, and they did not return during the course

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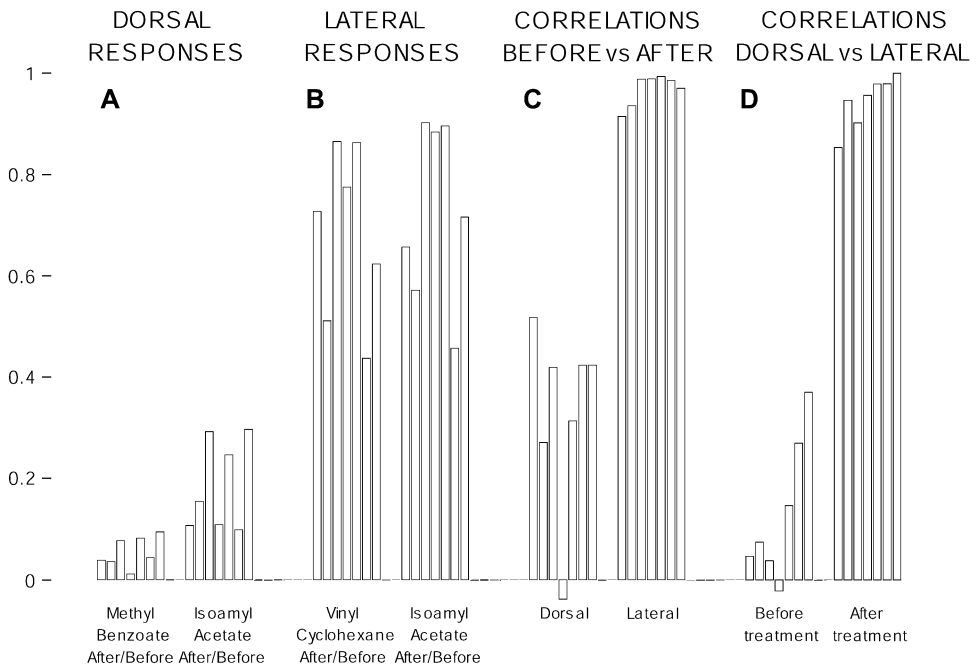

Figure 4 Changes in properties of the dorsal and lateral responses after long-duration methyl propionate treatment (described in the text). Part **(A)** on the left shows the ratio of the peak dorsal EOG to the highest flow rate and concentration of methyl benzoate before treatment versus after treatment. Each bar represents one of the six rats with successful depression of the dorsal response. The right side of part A shows the same ratio for the isoamyl acetate response at the dorsal site. Part **(B)** shows similar ratios for the vinyl cyclohexane and isoamyl acetate responses at the lateral site. Part **(C)** shows the correlation between the responses to all odors at each set of concentrations and flow rates before treatment versus after treatment. This correlation was low for the dorsal site indicating some change in the order of responses, but it was very high for the lateral site. Part **(D)** shows a low correlation of the dorsal versus lateral responses before treatment, indicating that they were not very similar, but a high correlation after treatment, indicating that the small remaining dorsal responses had a very similar response pattern to the lateral responses.

of the experiment. Presentation of this stimulus to the opened epithelium abolished responses everywhere (data not shown), so we assume that the restricted damage to dorsomedial responses is because most of the odorant passes through the dorsomedial recess. Similar global destruction of odor responses was seen if methyl propionate was presented at high flow rates (500 ml/min). The response decrements were probably due to damage to the tissue rather than long-term adaptation because long-duration methyl benzoate stimulation that produced responses of the same size did not permanently destroy responses.

Localized damage of the dorsomedial response was successful in six rats as shown by the change in response size and the profile of peak responses to the different odorants in our battery before and after the methyl propionate treatment. We measured the change in response size by comparing the responses before and after treatment. Responses to methyl benzoate and isoamyl acetate on the dorsal site were strongly decreased by the treatment (Figure 4A). The modest decrease in the lateral responses to isoamyl acetate and vinyl cyclohexane (Figure 4B) was probably rundown in the response over time. The pattern of response across the series of 27 stimuli (odors × concentrations × flow rates) was assessed by testing the correlation of EOG peak sizes. This pattern of correlation across the odorant battery was high for the lateral responses before versus after treatment. In contrast, pattern of dorsal responses did change, so that the before versus after correlation was substantially lower (Figure 4C). Most importantly, the pattern of dorsomedial responses were very different from the lateral responses before treatment (i.e., the correlation between the two was low). After treatment, the residual dorsal responses correlated very highly with the lateral responses (Figure 4D). The very high correlation between the small residual dorsal responses and the lateral responses indicates that the dorsal responses were simply the result of volume conduction. In four animals, this procedure was judged inadequate because there was not sufficient depression of the dorsal response compared to the lateral response. Therefore, only the seven rats in Figure 4 were included in the analysis of latency and slope. For those seven animals, the ratio of depression of the dorsal methyl benzoate response (after/before) divided by the ratio of depression of the lateral vinyl cyclohexane response ranged from 0.015 to 0.152 (mean 0.078).

After successful methyl propionate treatment, the latency of lateral EOGs usually became longer. Figure 5 shows an example where treatment reduced the dorsomedial response by about 90% and the lateral response by 33%. After treatment, lateral latency at 500 ml/min was unchanged, while the latencies at 100 and 200 ml/min were longer. All of the lateral

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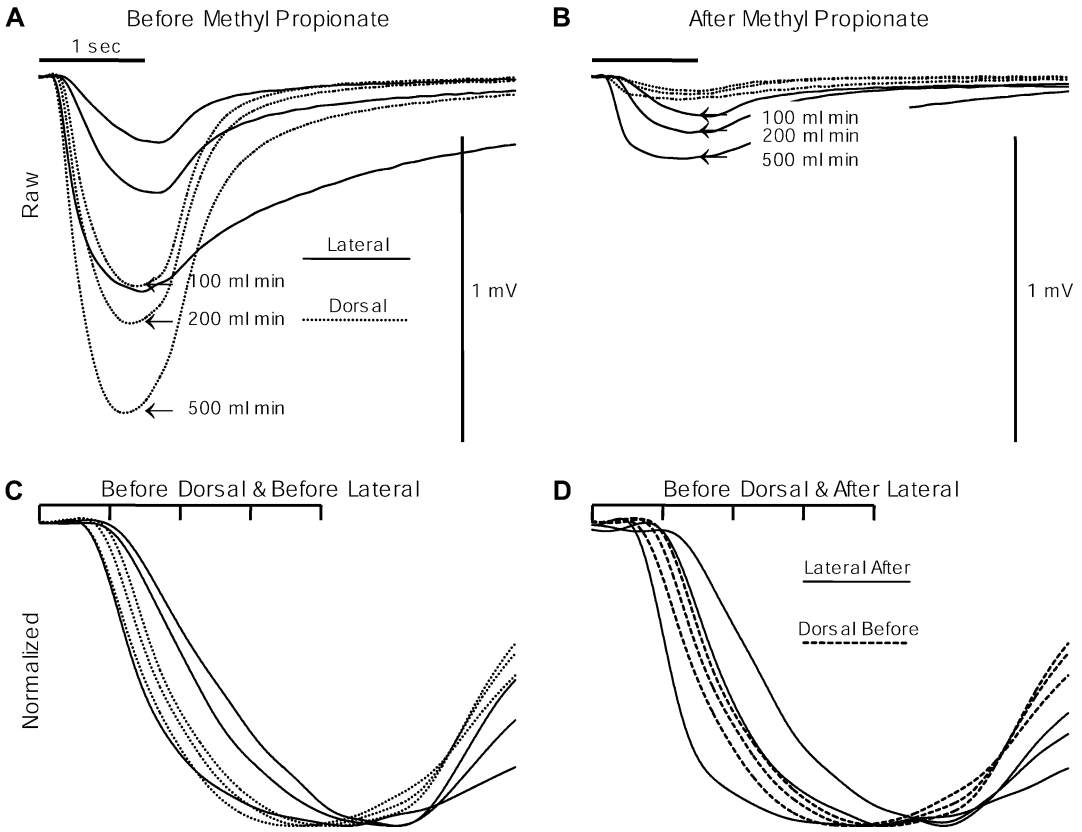

Figure 5 Long-duration methyl propionate treatment decreased the size of the dorsal response size and simplified the waveforms of the lateral response. Solid lines are the lateral responses and dotted lines are the dorsal responses to an isoamyl acetate stimulus (0.001 times vapor saturation). The upper records show the response magnitudes and full time course before **(A)** and after **(B)** methyl propionate treatment. Panel **(C)** shows the response before treatment, normalized with an expanded time base (time bar 1 s divided with 250 ms markers). Two responses were averaged for each record. Panel **(D)** compares normalized records of the dorsal responses before treatment (heavy dashed lines, the same records as in part panel C) and lateral responses after treatment (solid lines). Note that the responses at the highest flow rate leave the baseline at similar times the lateral responses at lower flow rates lag behind the dorsal responses.

responses became steeper. The lateral latencies were increased at the lower flow rates.

Figure 7 summarizes the latencies for the seven animals described in Figure 4. The dorsal responses before treatment are compared to the lateral responses after removal of the dorsal response. Overall, the lateral latencies were longer than the dorsal latencies, and the difference between the dorsal and lateral latencies increased as the airflow slowed. The overall mean latency differences at 100, 200, and 500 ml/min were 83, 36, and 9 ms, respectively. The dorsal latencies tended to be shorter for the odorants with larger Hansen solubility parameters. This effect was dominated by the longer latencies for the vinyl cyclohexane response and might disappear if it were possible to match the response sizes for all the stimuli. The latencies for vinyl cyclohexane are at the highest flow rate, and the latencies for isoamyl acetate and vinyl cyclohexane were not significantly different between sites. This indicates that the differences at the lower flow rates were not due to damage to the lateral sites by the methyl propionate treatment.

We also compared the slopes of the responses for the seven animals shown in Figure 4. The shapes of these responses were not easily characterized by a single value (see Figures 3 and 5). However, the initial slope to the 50% point was reasonably well fit by the template illustrated in Figure 6. We used the slope of that template to estimate the initial slopes of the responses. Figure 8 shows that the initial slopes depend strongly on nasal flow rate. The mean slopes for the dorsal site increased with increasing water solubility while the mean slopes for the lateral site decreased with increasing solubility. The slopes increased with flow rate but were not significantly affected by concentration. The change in response slope from the lowest to highest flow rate did not change across odorants for the lateral site, but the difference across odorants was significant for the dorsal site ($P < 0.01$ and $N = 6$ by the Friedman test). The only significant difference was between methyl benzoate and vinyl cyclohexane ($P < 0.01$ by the Scheffé procedure for multiple comparisons).

As suggested by the results of Figure 4, the methyl propionate treatment had no effect on the relative response magnitudes for the lateral site. A plot comparing the dorsal response before treatment to the lateral response after treatment would be indistinguishable from Figure 2. This

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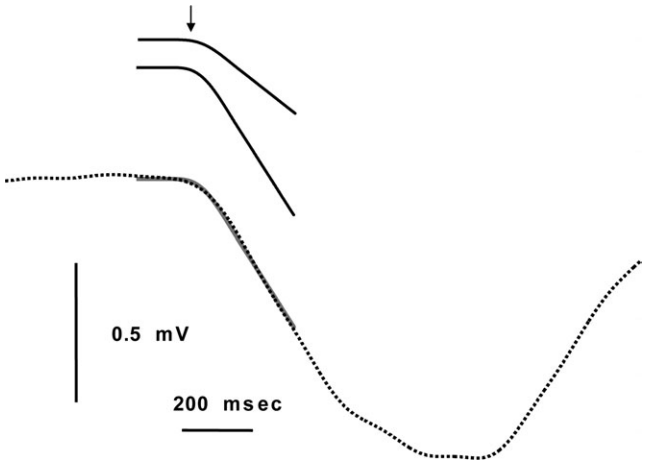

Figure 6 Illustration of the latency and slope measurement in response to vinyl cyclohexane at 100 ml/min and 0.001× saturation. The dotted line is the EOG recorded from the lateral site. The solid lines above the trace are examples of the template that was matched to the baseline and initial 50% of the response. The time and slope of the template were manipulated to the point of least squares fit. The fit of the template to this response is shown in gray. Latency was recorded at the point of greatest change in the slope of the template, indicated by the arrow. This procedure did not attempt to fit the entire rising phase, which would have required a higher order.

indicated that, at least with long-duration stimuli, the volume conduction of dorsal responses did not affect the lateral response magnitudes and that they are a reasonable measure of the response of the epithelium. Unfortunately, we were not able to selectively destroy the lateral response, so we cannot evaluate the effect of lateral responses on dorsal responses. However, since dorsal responses had shorter latencies, that effect was probably minimal.

Discussion

Relation of response to odorant properties

We have chosen to describe the odor stimuli in terms of the Hansen solubility parameter because that was a useful descriptor in application to a larger body of our data (Scott, 2006). While other empirical descriptors, such as chromatographic retention time (Mozell *et al.*, 1991) are also very useful, these data are not always available from the literature for a peculiar compound. We will describe the stimuli along a hydrophilic to hydrophobic continuum as a short hand but caution that other factors influence entry into the mucosa (Hahn *et al.*, 1994; Zhao *et al.*, 2006). Only four odorants were used in order to test a series of concentrations and flow rates. These stimulus sets were chosen to span overlapping ranges of response and to be sure that the dynamic range of responses was not saturated. This is the particular reason that three concentrations of vinyl cyclohexane were used. The odorants were picked after a broader preliminary sampling of other stimuli (among them in order of ascending solubility parameter: heptane, alpha-terpinene, limonene, carvone, phenyl acetate, benzaldehyde, and acetophenone) which showed similar distribution of response on the dorsal and lateral sites and similar patterns of sensitivity to flow rate.

The responses to odorants in the intact rat olfactory epithelium are affected by nasal flow rate and that effect is related to the polarity of the odorant stimuli. The responses to long-duration stimuli presented at high flow rates to the intact nasal cavity in Figure 2 show a similar distribution to the responses of the exposed epithelium in Figure 1. This is in line with previous findings that the responses to odorants vary across the olfactory epithelium or olfactory bulb in relation to their physical properties. However, the responses to hydrophilic odorants are somewhat smaller for the intact preparation, even though they are normalized to a standard stimulus. This decrease for hydrophilic odorants is more evident with lower flow rates. These data stress the issue of receptor access for hydrophilic odorants, which tend to be sorbed to the nasal surface before they reach the receptor sheet (Mozell *et al.*, 1991). These nasal flow rates include the range of rates of inspiration in rat sniffing behavior (Youngentob *et al.*, 1987).

The anatomy of the nose and the airflow models (Kimbell *et al.*, 1997; Zhao *et al.*, 2006) suggest a slower airflow in the lateral recess. Our earlier reports (Ezeh *et al.*, 1995; Scott-Johnson *et al.*, 2000) had supported this conclusion by finding longer latencies and lower slopes in the lateral recess. We recently became concerned about this interpretation on the basis of recordings like those of Figure 3 that appeared to show short-latency lateral response. The experiments with methyl propionate treatment of the dorsal responses have allowed quantification of the latencies and show a statistically reliable difference between the dorsal and lateral response onsets. These differences are as much as 80 ms for low flow rates, but they were smaller at the highest flow rates corresponding to the most vigorous sniffing. Since the high flow rate latencies for the most hydrophobic odorants were not significantly changed between the dorsal site before the methyl propionate treatment and the lateral site after treatment, it is unlikely the large latency differences for the lower flow rates resulted from damage to the lateral site by that treatment. Therefore, the current data strongly support the model of slower airflow in the lateral recess of the rat nasal cavity.

Analysis of rise times was proposed by Scott-Johnson *et al.* (2000) as evidence in favor of greater sorption of hydrophilic odorants. The variations in waveform reported here call those rise time measurements into question. We could not adequately measure rise times on all of our responses in the current study because not all the responses reach peak value. However, the initial slope responses were not affected by this problem, and they showed systematic changes related to flow rate and to odorant. At the dorsal site, the slopes were systematically steeper for more hydrophilic odorants; this is in line with the faster removal of hydrophilic odorants from

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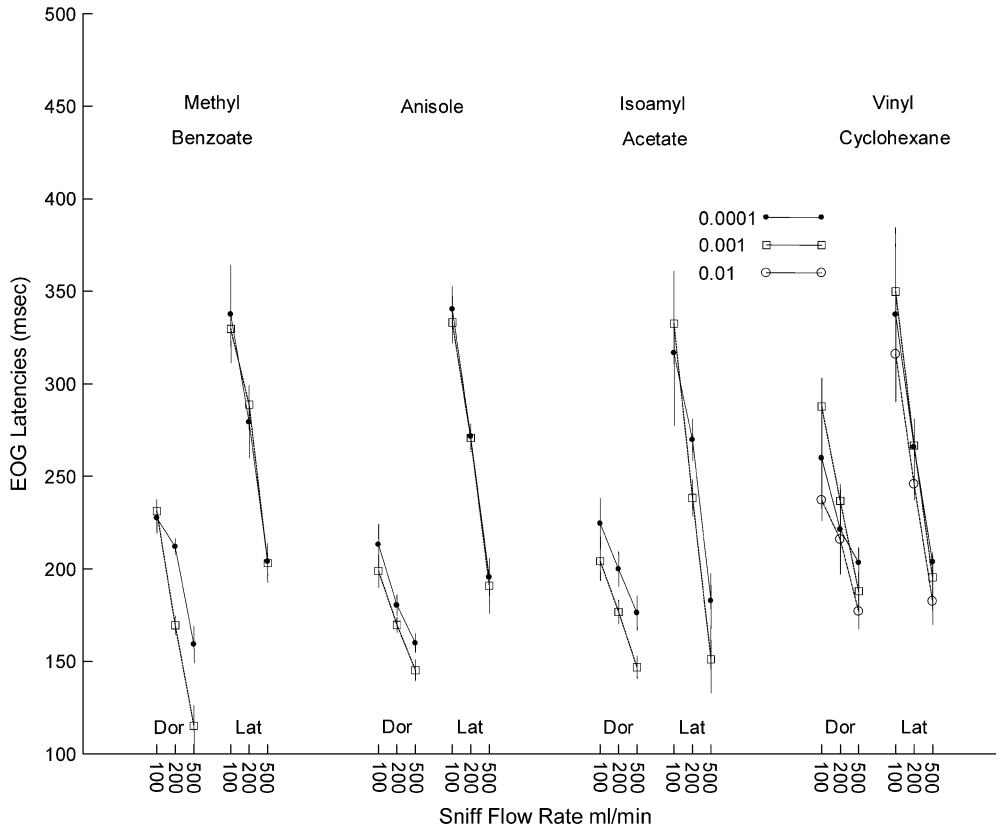

Figure 7 Latencies (mean ± SEM, $N = 7$) of dorsal responses before methyl propionate treatment versus lateral latencies after treatment for the animals illustrated in Figure 4. The lateral latencies across odorants and flow rates are longer at the lateral site ($P < 0.001$ by the rank sums test, using six animals and the higher concentrations because of missing values for the lowest flow rate). The differences among odorants for the lateral minus dorsal latencies were significant after averaging across concentrations and flow rates ($P < 0.01$, $N = 5$). Effects of flow rate averaged across odorants at the highest concentration were significant on the dorsal sites ($P < 0.01$, $N = 7$) and on the ventral sites ($P < 0.01$, $N = 6$). The size of the dorsal minus lateral latency difference changes significantly across the three flow rates at the highest concentration ($P < 0.01$, $N = 6$). At the highest flow rates, the latencies for isoamyl acetate or vinyl cyclohexane were not significantly different between the two sites at any of the concentrations ($P > 0.05$ by the rank sums test for each comparison, $N = 7$ in each case). For each of those comparisons, the difference was less than 8 ms.

a rapidly moving air stream. At the lateral site, the response slopes were steeper for more hydrophobic odorants. This suggests a slower arrival of odorant at the receptors, perhaps because it takes time to saturate the upstream sorption of those odorants. In any case, the slopes at the two sites have strongly different tendencies. In fact, the correlation across the mean value of dorsal minus lateral slopes for the four odorants with the Hansen solubility parameter has a value of 0.79 for the seven animals at the two highest flow rates ($P < 0.01$). While the EOG waveform should be interpreted with great caution, the fact that responses to hydrophilic odorants developed more slowly in the lateral recess than in the dorsal recess is consistent with the interpretation that saturation of upstream sorption of these odorants is necessary before the full response develops. This is what one would expect from the chromatographic model (Mozell *et al.*, 1991).

The present results provide reassurance about the use of the EOG to estimate the response of these different regions of the epithelium in intact animals. The results for the peak voltage at the lateral sites in these recordings were not different before versus after extensive damage to the dorsal sites. This indicates that, at least for long-duration stimuli, it is possible to record EOG response magnitudes that are not greatly compromised by responses at other sites. We suggest that reasonable criteria for judging the viability of such responses would be 1) that responses at both dorsal and lateral sites should be at least 1 mV in response to a strong stimulus like isoamyl acetate at 0.01 time saturation, 2) there should be greater dorsal responses to hydrophilic odorants coupled with greater lateral responses to hydrophobic odorants, and 3) low flow rates in the order of 100–200 ml/min should produce a visible latency difference for odorants, such as isoamyl acetate, that activate both sites strongly. Recordings that do not fulfill these criteria should be treated with caution as they may be contaminated by volume conduction. The EOG can be a valuable tool but like many such tools it is subject to validation.

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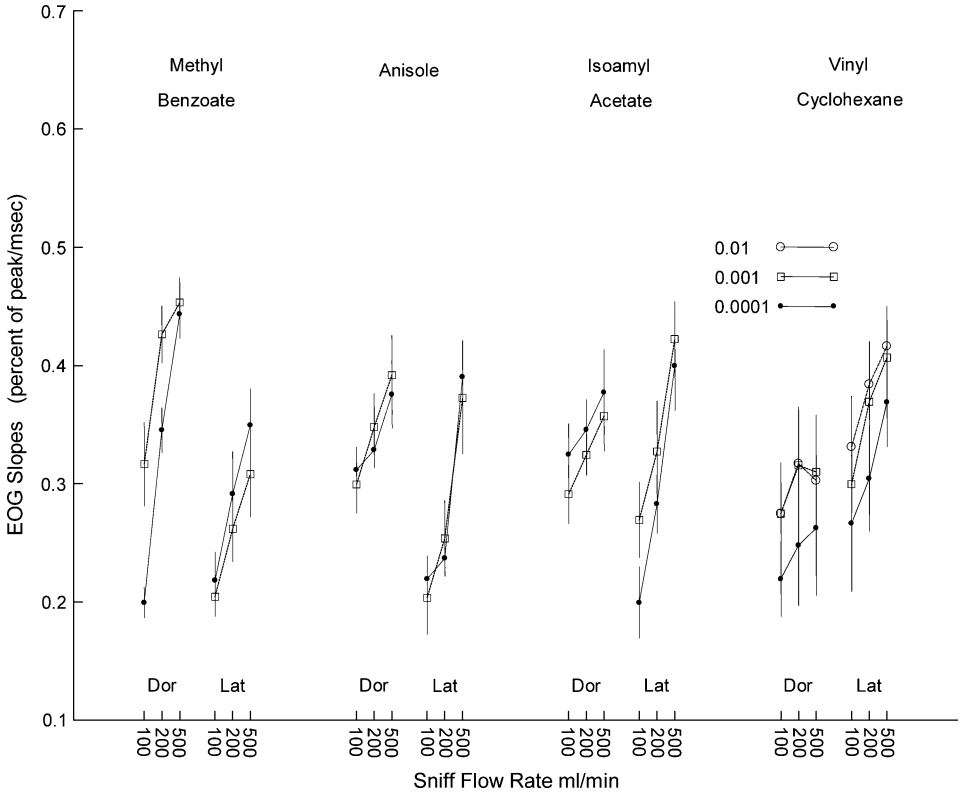

Figure 8 Initial slopes (mean ± SEM, $N = 7$) of dorsal responses before methyl propionate treatment versus lateral initial slopes after treatment for the animals illustrated in Figure 4. For the upper two flow rates, there were significant effects of odor for the mean slopes of the dorsal responses, of the lateral responses, and of the difference between the dorsal and lateral responses (all $P < 0.01$, $N = 7$ by the Friedman test). There was a similar outcome looking at all flow rates for dilutions above 0.0001 ($P < 0.01$ for the comparisons of dorsal or ventral slopes across odorants and $P < 0.05$ for the difference between dorsal and ventral slopes across odorants by the Friedman test, $N = 6$). There were significant effects across flow rate for the dorsal slopes ($P < 0.01$, $N = 7$) and the ventral slopes ($P < 0.01$, $N = 6$) by the Friedman test (excluding the lowest flow rate to avoid missing values).

Implications for sensory processing

These data support the concept that the relationship between flow patterns and receptor properties optimizes the efficiency of response (Schoenfeld and Cleland, 2005, 2006; Scott, 2006). The receptors most sensitive to hydrophilic odorants are placed where an increase in sniff velocity can bring more of the hydrophilic odorants to the sensory sheet. Receptors most sensitive to hydrophobic odorants are placed where the airflow is slow, favoring transfer to the mucosa.

The relationship between hydrophobicity and the effect of flow velocity is striking. Independently of site, the most hydrophobic odorant had a nearly flat response to velocity. The most hydrophilic odorant had the steepest velocity response at the dorsal site. The two odorants with intermediate properties had the steepest velocity response at the lateral site. This is probably because very little methyl benzoate reached the lateral site. Scott-Johnson *et al.* (2000) found a similarly small lateral response to benzaldehyde and carvone, which have properties similar to methyl benzoate. They also found the lateral isoamyl acetate response very velocity sensitive, while limonene and benzene responses were less strongly affected by velocity. It is likely that an organism may manipulate the sniff velocity to optimize response to odors or components of a complex odor in a manner as suggested by human responses (Sobel *et al.*, 2000). We should also note that the measurements here are for very long-duration stimuli, and the effects of velocity may become even greater for some stimuli at shorter durations.

The latency and response slope observations may have implications for coding mechanisms in the olfactory bulb. Anatomical and physiological studies have demonstrated mechanisms that may compare activity at significant distances in the olfactory bulb (Schoenfeld *et al.*, 1985; Belluscio *et al.*, 2002; Lowe, 2002; Xiong and Chen, 2002; Augnst *et al.*, 2003). This latency difference suggests that any mechanisms that compare responses across a significant expanse of the olfactory bulb will be affected by the times of arrival of inputs from the bulb. Whether these delays could be a positive cue for temporal coding or would be a source of noise that must be suppressed is a matter for future research. It is also possible that a high velocity sniff would minimize the latency difference between the lateral and medial recesses, so that optimal processing would occur.

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As observed by an anonymous reviewer, these considerations of latency may not apply to the region of the septum and medial wall of the turbinates expressing zones 2–4. The published models do not suggest slower airflow for the parts of the medial recess corresponding to these zones. Although we have extensive data from this region for the exposed epithelium (Scott and Brierley, 1999; Scott *et al.*, 2000), recording EOGs from this region in an intact animal is problematic. The ORNs in this region send their axons to medial glomeruli (Schoenfeld *et al.*, 1994), and it would be of great interest to know whether the temporal characteristics of their activation are similar since the medial and lateral glomeruli associated with a particular molecular receptor are reciprocally connected (Schoenfeld *et al.*, 1985; Belluscio *et al.*, 2002).

Acknowledgements

We thank William Goolsby for LabView programming and Fredrick Schmidt for help with electronics. This work was supported by grant number DC00113 from the National Institutes of Health.

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Accepted May 9, 2006

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