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Effects of Air Flow on Rat Electroolfactogram

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Abstract

The electroolfactogram (EOG) previously has been used to demonstrate the regional distribution of rat olfactory epithelial odorant responses. Here, we evaluated the effects of airflow parameters on EOGs in two preparations: one where odorants were directly applied to the epithelium (opened preparation) and one where odorants were drawn through the nasal passages by an artificial sniff (closed preparation). EOG rise times served as one measure of odorant access. For isoamyl acetate (but not for limonene), rise times were slower in the lateral recesses of the closed (but not the opened) preparation. Polar odorants (amyl acetate, carvone and benzaldehyde) evoked smaller responses in the closed preparation than in the opened preparation, and these responses were particularly depressed in the lateral regions of the closed preparation. Responses to nonpolar hydrocarbon odorants (limonene and benzene) were equal in the lateral regions of both preparations, but were somewhat depressed in the medial region of the closed preparation. The responses to some polar odorants in the closed preparation were sensitive to changes in airflow parameters. These data suggest that the sorptive properties of the nose contribute substantially to determining the response of the epithelium and act to increase differences produced by inherent receptor mechanisms.

Introduction

Odor discrimination depends on selectivity of the receptors and on processes that govern access to the receptor neurons. In his 1976 review, Moulton distinguished between inherent properties, referring to receptor selectivity to odors, and imposed properties, referring to physical chemical principles related to odorant access (Moulton, 1976). There is clear evidence for inherent spatial patterns of odorant response in several species (Kauer and Moulton, 1974; Mackay-Sim and Kubie, 1981; Mackay-Sim *et al.*, 1982; Mackay-Sim and Kesteven, 1994; Youngentob *et al.*, 1995; Scott *et al.*, 1996). Scott *et al.* (Scott *et al.*, 1997) claimed that these spatial distributions in adult rats correlate with the pattern expression zones of olfactory receptor genes observed in neonatal rats and mice (Ressler *et al.*, 1993; Vassar *et al.*, 1993), suggesting that the distributions may reflect different receptor populations. In order to test receptor neuron sensitivity to various odorants, those electroolfactogram (EOG) studies directly exposed the epithelium to odorants in an attempt to address the issue of restriction of odorant access by the gross topography of the nasal cavity.

The properties of the nasal cavity that govern access of odorant molecules to the receptor neurons—the imposed properties of Moulton (Moulton, 1976)—are also very important in determining spatial response patterns. Drawing from the analogy with gas chromatography, Mozell and his colleagues used the frog (Mozell, 1970; Hornung and Mozell, 1977; Mozell *et al.*, 1984, 1991) and rat (Kent *et al.*, 1996) to show how physical–chemical and sorptive properties govern access to the receptor neurons. They proposed that the degree to which odorants partition into the mucus and diffuse to receptors determines this access (Keyhani *et al.*, 1997). As odors are drawn along the mucosal surface, polar odors tend to be removed from the airstream. In the frog nose, the response to the polar odorant carvone was reduced downstream while the response to the nonpolar odorant octane was unaffected (Mozell, 1970). In addition to these chemical properties, the flow rates of the odorant through the system are important (Mozell *et al.*, 1991). The downstream response to polar odors will increase as flow rate increases because some of the upstream sorption sites are occupied. However, nonpolar odors are affected in another way. If the airstream flow rate is increased without increasing the number of molecules per second, then nonpolar odors are more poorly sorbed into the tissue and their access to receptors is reduced (Mozell, 1970; Mozell and Jagodowicz, 1973; Hornung and Mozell, 1981; Mozell *et al.*, 1991).

We studied this issue in the rat using the EOG as a response measure. We compared responses in a closed preparation with an artificial sniff to a preparation in which the nasal cavity was opened and odorants directly applied to reduce imposed influences (i.e. sorption by upstream

tissues). Our rationale came from previous studies in both opened (Scott *et al.*, 1997; Scott and Brierley, 1999) and closed preparations (Ezeh *et al.*, 1995), showing that, for several odorants, the profiles of response magnitudes changed significantly when the electrodes were moved across regions corresponding to different receptor gene expres- sion zones. On the other hand, the profiles for a particular odorant changed very little for different points along the anterior–posterior axis parallel to the gene expression zones. The most dorsal expression zone corresponds to the dorsomedial recess of the nasal cavity. The other zones are represented successively more ventrally and laterally along the midline and in the lateral recesses (Ressler *et al.*, 1993; Vassar *et al.*, 1993). The proportion of the olfactory epithelium in the lateral recesses is quite large in the rodent (Clancy *et al.*, 1994). We have therefore chosen to compare responses between the dorsomedial recess and the extreme lateral recess, but have included comparisons along the anterior–posterior axis to confirm our previous observations.

The chromatographic hypothesis presupposes that there is a gradient of tissue over which the sorptive process can act. Unlike the frog model (Mozell, 1970) or the study conducted along the medial surface of the rat epithelium (Kent *et al.*, 1996), the portions of the epithelium that we have studied are not linearly arranged. We are proposing that the airflow is slower in the lateral recesses of the rodent epithelium because of a large cross-sectional area and narrow lumen. This slower airflow would increase sorption, just as increasing distance along a linear pathway would increase sorption. Modeling of airflow in the rat nose supports this hypothesis of slower airflow in the lateral recess (Kimbell *et al.*, 1997). Observations that EOG latencies were systematically longer in the lateral recess of the rat than in the dorsal recess (Ezeh *et al.*, 1995) are also consistent with this proposition.

Comparison of recordings in the lateral recess of the closed preparation (Scott *et al.*, 1996) and the ventral region along the midline of the opened preparation (Scott *et al.*, 1997, 2000; Scott and Brierley, 1999) shows that the responses to polar odorants such as carvone or benzaldehyde were smaller in the closed preparation. However, those data are of limited use for evaluating the sorption hypothesis because of the normalization procedures and the exact position of the recordings.

In addition to comparing the responses in the opened and closed preparations, we attempted to test the sorption effects in two other ways. We have manipulated the duration and flow rate of odor pulses to test the predictions from the chromatographic hypothesis outlined above. We have also measured the rise time of response to isoamyl acetate and limonene. These two odorants gave large responses in most animals under all conditions or recording. We expected to see that the isoamyl acetate responses would develop more slowly in the closed preparations, particularly in the lateral recess, and that there would be little difference between the two preparations on the limonene rise times. This would serve as additional evidence that sorption along the flow path was regulating odorant concentration near the receptor neurons.

Materials and methods

General surgical procedures

Male Sprague–Dawley rats (200–500 g) were rapidly killed with a cocktail of 87 mg ketamine, 13 mg xylazine and 0.3 mg butorphanol/kg body wt or 0.1 cm^3/100 g body wt or Nembutal (50 mg/ml). This prevented bleeding in the extensive surgery of the opened preparation and also maintained maximal patency of the nasal airway. Two surgical preparations were used and will be referred to as the opened preparation and the closed preparation. For the closed preparation only, animals were pretreated with atropine sulfate (0.06 mg) for 30 min prior to injection.

For the opened preparation, the right eye, the skin over the nasal cavity, the zygomatic arch, and the turbinate bones on the right side were removed along with the anterior part of the orbital surface. A portion of the frontal bone was removed to expose the dorsal lateral surface of endoturbinate II (Figure 1A).

For the closed preparation, we drilled through the bone and inserted electrodes through the epithelium to the luminal surface. This approach was described previously by Ezeh *et al.* (Ezeh *et al.*, 1995) and Scott *et al.* (Scott *et al.*, 1996). The rat olfactory epithelium covers a complex series of bones. The two epithelial sites most easily accessible by penetration from the outside are the dorsal medial recess and the extreme lateral recesses. We chose the space between the bases of ectoturbinate II and endoturbinate II (Figure 1B) for the lateral recording. This lateral recess is probably not entirely covered by olfactory epithelium, but an elec-

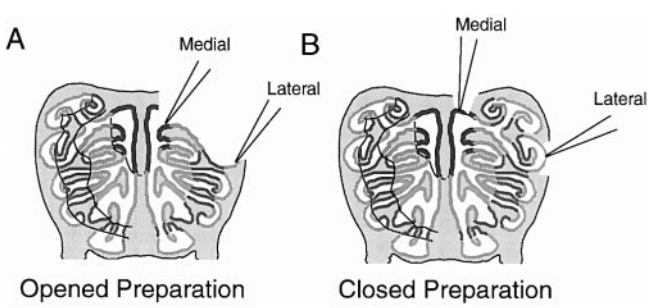

Figure 1 Panel **A** represents a schematic of electrode placements on the dorsal surface of ectoturbinate II for electrodes recorded simultaneously in the medial and lateral sites of the opened preparation. Panel **B** shows a cross-section with typical recording sites in the closed preparation for electrodes placed in the medial and lateral regions. Two electrodes were placed in each region—one in an anterior position and the other in a posterior position. The four electrodes were recorded simultaneously. The shading indicates the approximate positions of the olfactory receptor gene expression zones of the rat drawn from the observations of Vassar *et al.* (Vassar *et al.*, 1993). While we cannot ensure that the electrode positions were exactly the same in the two preparations, the medial and lateral electrodes should have been in equivalent zones in the two preparations.

trode inserted here could encounter only the two most ventral expression zones. A single cannula was inserted up the trachea to end over the soft palate. The cannula was connected to a valved vacuum line to establish an artificial sniff. During surgery and between stimulus presentations, a 2 s (50% duty cycle) sniff was maintained to keep the airway clear.

Odorant stimulation

Odorants

The odors used were isoamyl acetate (an aliphatic ester), limonene (a terpene hydrocarbon), carvone (a terpene ketone), benzene (an aromatic hydrocarbon) and benzaldehyde (an aromatic aldehyde). We chose these odorants to maximize the diversity of chemical structures and because of their effectiveness in evoking EOG epithelial responses in previous studies (Scott *et al.*, 1996, 2000).

Odorant delivery

Odorants were introduced through an 8 mm glass tube with nine ports. A background flow of clean, humidified air (1000 ml/min) was maintained in this tube. Air saturated with one of the test odorants was injected via the ports at a rate of 100 ml to produce dilutions of 10^{-1} from saturation. (Weaker dilutions were used in some of the experiments, but we have reported only the stronger stimuli here because the lower concentrations of some stimuli failed to produce reliable responses at the lateral sites.) The odorant source bottles had a volume of 100 cm^3 and the odorant chemical presented a surface of ~4 cm^2 on the bottom. The bottles were fitted with Teflon stoppers and tubes for connection to the syringe pump and odorant stimulus tube. There was a minimum inter-stimulus interval of 1.5 min during standard runs. Stimulation procedures were automated through the use of a BASIC computer program. Most recordings consisted of several runs of 5–9 odorants, including a blank, at a single concentration. For stimulus presentations for the opened preparations, each odorant was introduced in a single 0.6 s sniff. The computer imposed a period of 10 s with no sniff just before the stimulus to ensure that the odorant had reached the desired concentration.

Stimulation in the two preparations

The stimulation procedures were slightly different for the two preparations. For the opened preparations, the end of the glass odorant tube was fitted with a glass T-tube with the side tube connected by a valve to the vacuum line. The tip of the T-tube was placed ~1 cm from the surface of the epithelium. The vacuum line was used to draw away odorants for 10 s just before stimulation. This period was usually sufficient to allow odorant concentration to rise to the maximum effect. However, in a small number of cases the response to some odorants did not peak by the end of this period (see Results). Odorant application was produced by turning off the vacuum for a period of 0.6 s.

For the closed preparation, the T-tube was removed and the end of the odorant tube was placed loosely around the animal's nares. The 1000 ml/min airflow in the odorant tube always exceeded the maximum sniff flow (600 ml/min) to avoid pulling room air that might contain residual odorant into the nose. The artificial sniff was stopped for the 10 s period before each sniff while concentration was building up in the odorant tube. Odorants were presented for either of two durations. We refer to durations of 0.22 s as 'SHORT' and 2.4 s as 'LONG'. The flow rate of the air through the nose during a sniff was either 200 ml/min (LOW) or 600 ml/min (HIGH).

Data collection

Recordings were made with glass micropipettes filled with Ringer's solution, broken to a resistance of 0–8 MΩ. The leads from these electrodes were connected to two Axoclamp 2-channel amplifiers. An indifferent electrode was placed on the parietal bone. For the opened preparations, four electrodes were aligned with equal spacing medial-to-lateral along the dorsal lateral surface of endoturbinate II, for the purposes of this experiment we compared the extreme medial and lateral electrodes (Figure 1A). A computer-generated auditory signal proportional to electrode voltage was used to determine initial contact with the epithelial surface. For the closed preparation, the electrodes were driven carefully through the epithelium until a large negative response to an isoamyl acetate stimulus was encountered. In most cases, we were able to place one electrode in an anterior position and one in a posterior position in both the dorsal medial and the lateral sites to determine if there were anterior to posterior response differences within the same region. These anterior and posterior electrodes were usually separated by 2–4 mm in the lateral region and 3–6 mm in the dorsal medial region. The positions of all electrodes were recorded photographically.

The outputs were displayed on an oscilloscope and fed by an A–D converter to a computer that digitized the traces at 6 Hz. The digitized traces were displayed off line for quality control. Records with excessive drift were rejected. Occasional A–D conversion artefacts or noisy records were edited out. The peak negative voltage was computed relative to the baseline just before the stimulus and stored in a file for statistical analysis.

Unlike the other reports from this laboratory (Ezeh *et al.*, 1995; Scott *et al.*, 1997; Scott and Brierley, 1999), the data were not normalized. Although normalization reduces extraneous variance due to differences among animals and to the depth of electrode penetration, it requires using a standard odorant that may itself be subject to attenuation by sorption onto the tissue. As we will show here, the isoamyl acetate odorant that we have commonly used as a standard in other experiments with direct odorant application is strongly subject to airflow effects in the closed preparation.

Data analysis

For most comparisons, we averaged the data for the two medial electrodes and two lateral electrodes for each animal in the closed preparation. These averages were compared to the single medial and lateral electrodes of the opened preparations (see Figure 1). We justify this procedure because, although the average posterior responses were smaller for all odorants, the anterior–posterior differences were much smaller than the medial–lateral differences. This average of two points gave us a measure that reduced variability resulting from differences such as the electrode depth in the epithelium or trauma to the epithelium from penetration. As noted above, these recording sites were roughly comparable to the single medial and single lateral recording sites in the opened preparation. All statistical comparisons were made with nonparametric tests because of the small numbers and the lack of normal distributions.

We have focused on responses obtained at the highest concentration. This was because the two highly polar odorants (carvone and benzaldehyde) did not produce measurable responses at the lateral sites at lower concentrations. In fact, these responses were usually not different from the blank, even at the highest concentration. Therefore, we limited the subsequent data collection and analysis to the higher concentrations.

Rise times were calculated as the time from 10 to 90% of the maximum peak voltage. A polynomial fit to the points was used to interpolate between the digitized points. Some slowly developing responses never reached a maximum. This fact actually tended to underestimate the differences that we report.

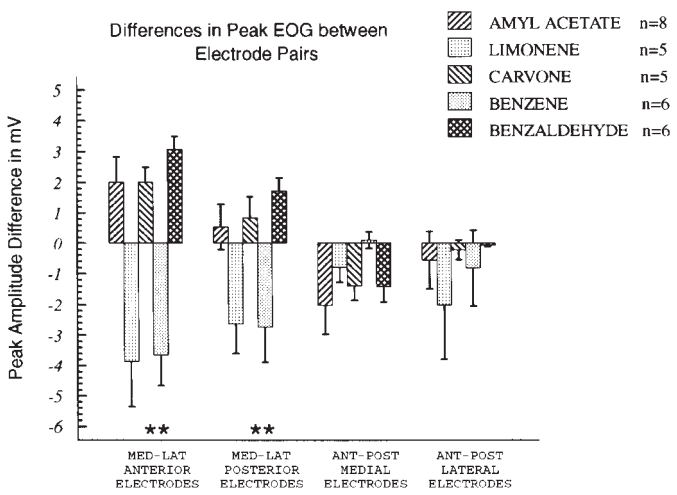

Figure 2 Differences in peak EOG voltages (mean ± SEM) for the pairings of the four electrodes. For example, for the 'ANTERIOR MED-LAT' comparison, the average response to each odorant on the anterior lateral electrode was subtracted from the average response to the same odorant on the anterior medial electrode the same animal (n = number of animals for each odorant; $^{**}P > 0.01$ by the Kruskal–Wallis one-way analysis of variance across the five odorants for comparisons with a particular electrode pair).

Results

The closed preparation differences in response magnitude along the anterior-to-posterior axis were much smaller than the differences for medial-to-lateral comparisons. Figure 2 shows the averages across animals obtained by subtracting the peak voltage response on the pair of anterior electrodes and the pair of posterior electrodes. This figure was based on the data for the long-duration, high-flow-rate condition, but the same result was seen with the other sniff parameters. This result confirms the expectation that the largest differences would be found between the medial electrode (in the dorsal medial recess) and the lateral recess. It provides the justification for our combining the results of the two medial electrodes and the two lateral electrodes.

Figure 3 shows a set of simultaneously recorded medial and lateral responses to isoamyl acetate and to limonene presented with the four sniff conditions. These were odorants that evoked optimal responses in different regions and for which the responses behaved differently over the different sniff conditions. Even at the highest flow rate and

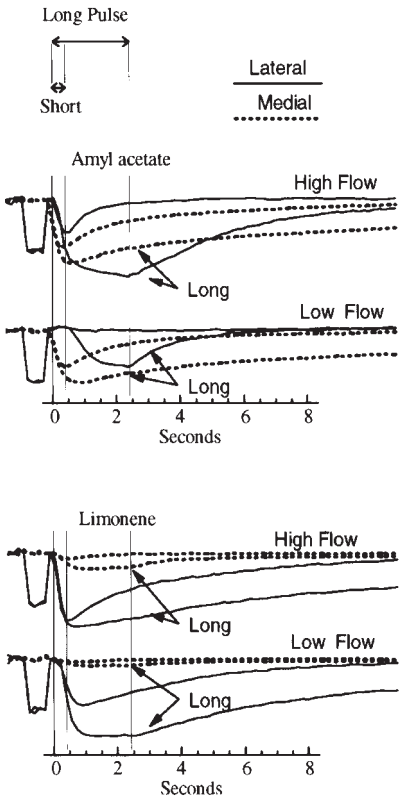

Figure 3 Response waveforms in the closed preparation to isoamyl acetate and limonene odorants diluted at 10^{-1} under the four conditions. The arrows point to the responses resulting from long stimulus durations (2.4 s). The solid waveforms depict responses to recordings in the lateral region and the dashed waveforms depict responses to recordings at the medial region.

longest stimulus duration, the limonene responses were much smaller at the medial site than at the lateral site. This confirms the regional differences in response previously shown with the opened preparation (Scott and Brierley, 1999). However, the isoamyl acetate responses were particularly sensitive to the parameters of the sniff. At the long duration, the isoamyl acetate responses were large at both medial and lateral sites, but at the short duration the peak lateral responses were smaller. The long duration stimuli allowed us to follow the temporal development of the response. In this example, the limonene response at the lateral site reached a peak in <1 s, while the isoamyl acetate response at the same site was still increasing in size at the end of the 2.4 s stimulus period. These time-course differences were not apparent at the medial site.

Figure 4A summarizes the rise times of responses to these two odorants for six closed preparations. This figure slightly underestimates the difference between the two sets of responses because, in some cases, the isoamyl acetate response did reach a peak even during the longer duration stimulus (as in Figure 3). The isoamyl acetate responses on the lateral sites were slower than the responses on the medial sites, but the limonene responses were not significantly different for the two sites. In contrast, for the seven opened preparations (Figure 4B), there were no significant differences in the rise times between medial and lateral electrodes for either of these two odorants. In the opened preparation, the limonene rise times were significantly slower than the isoamyl acetate rise times at the medial electrode.

The peak voltages measured in the opened and closed preparations for five odorants are illustrated in Figure 5. For the closed preparation, we used each of the four sniff parameters illustrated in Figure 3. The medial and lateral measurements in each case are the averages of the two medial and lateral electrodes, respectively, for each animal. Essentially the same results were obtained comparing individual lateral and medial electrodes, but the use of the means simplified the figure and analysis. The Kruskal–Wallis test for each of the odorant panels showed that there were overall significant differences among the means ($P < 0.01$). For most odorants, the average response in the opened preparation was significantly larger than the average response in the corresponding closed preparation, under any of the flow conditions. This was particularly true for the highly polar odorants, carvone and benzaldehyde. Indeed, the benzaldehyde and carvone responses on the medial sites were not significantly different from the blanks. However, the limonene and benzene responses at the lateral sites were not significantly smaller than in the opened preparations.

For some of the odorants, the sniff parameters were important determinants of the response size at particular sites. This is summarized in Table 1, where comparisons were made for various combinations of regions and sniff parameters. The two medial sites and the two lateral sites

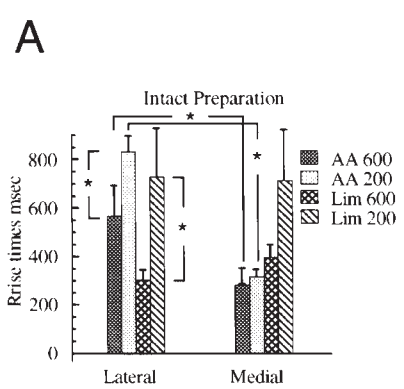

Figure 4 Panel **A** shows the average rise times (±SE) in the closed preparation for two odorants at the two flow rates when tested with the long stimulus duration. The data are for mean responses at the lateral site. Asterisks indicate responses at the medial site that were significantly faster than the response at the lateral and medial sites for the same odorant and flow rate ($P < 0.05$ by the Wilcoxon paired ranks test). Panel **B** shows the average rise times (±SE) in the opened preparation. The symbols show that the limonene responses were significantly slower ($P < 0.05$ by the Mann–Whitney *U*-test) than the isoamyl acetate responses at the medial site.

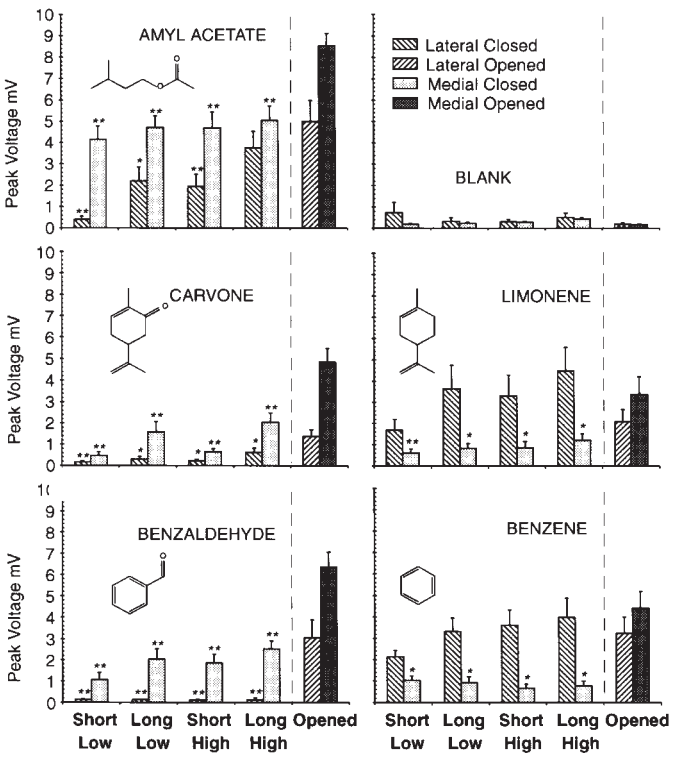

Figure 5 Average peak voltages (±SEM) for the opened preparation (at the right of each panel) and the closed preparations with the four flow parameters. Odorant structures are shown in each panel. The bottom two panels on the right show hydrocarbons with structures similar to the polar compounds on the left. Asterisks show significance levels of the comparison of the closed preparation with the corresponding site of the opened preparation (*$P < 0.05$; **$P < 0.01$ by the Mann–Whitney *U*-test). The numbers of animals for the closed and opened preparations, respectively, were: isoamyl acetate 8 and 10; carvone 5 and 8; limonene 5 and 8; benzene 6 and 3; benzaldehyde 6 and 8.

Table 1 *P* values for differences in responses due to sniff parameters for the closed preparation, i.e. stimulus duration and flow

Odorants	*P* values (lateral versus medial in closed preparation)	*P* values (differences in lateral response due to sniff)	*P* values (differences in medial response due to sniff)
Isoamyl acetate	0.01	0.01	n.s.
Carvone	0.01	–	0.05
Limonene	0.01	0.05	–
Benzene	0.01	n.s.	–
Benzaldehyde	0.01	–	n.s.

[a]Responses did not differ significantly from those observed for 'blanks' (control).

[b]n.s., not statistically significant.

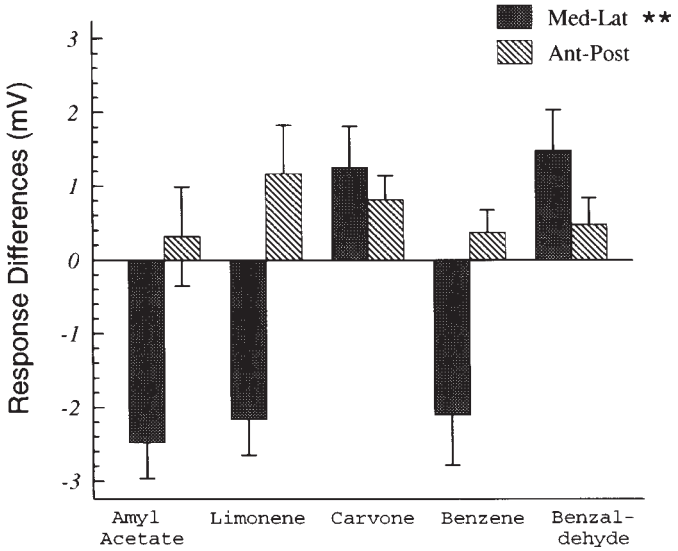

Figure 6 Relative effect of sniff parameter changes on medial–lateral electrode pairs and anterior–posterior electrode pairs. This figure compares the extreme sniff parameters, subtracting the response with SHORT–LOW pulses from the response with LONG–HIGH pulses. The bars show average differences (± SEM). **The distribution of differences across odorants for the medial–lateral electrode pairs is significant at $P < 0.01$ by the Kruskal–Wallis one way analysis of variance. The differences for the anterior–posterior electrode pairs are not significantly different across odorants.

were averaged for each animal as before. For each of the five odorants, there were significant ($P < 0.01$ by the Kruskal–Wallis test) differences among the set of four parameters over the two recording regions. However, when testing within either the lateral or medial regions, we could only find significant differences across parameters for isoamyl acetate and limonene in the lateral region and for carvone in the medial region (Friedman two-way analysis of variance). For carvone and benzaldehyde in the lateral region and for limonene and benzene in the medial region, such tests were not done because the responses were not significantly greater than the blanks.

These effects of changes in sniff duration or flow rate could also be assessed by comparing the differences in response magnitude as a function of these sniff parameters. For example, the medial-minus-lateral response difference for isoamyl acetate in Figure 5 becomes more negative as the sniff parameters progress from the SHORT–LOW to the LONG–HIGH conditions. This is also true for the limonene and benzene responses, but not for the carvone and benzaldehyde responses. Figure 6 illustrates the comparison of the extreme sniff parameters (SHORT–LOW versus LONG–HIGH) across the five odorants for the medial–lateral and anterior–posterior comparisons. The medial–lateral comparisons were significant at the $P < 0.01$ level by the Kruskal–Wallis test. The other sets of comparisons (SHORT–LOW versus SHORT–HIGH, LONG–LOW versus LONG–HIGH, SHORT–HIGH versus LONG–HIGH, and SHORT–LOW versus LONG–LOW) were all significant at $P < 0.05$. In contrast, the comparisons across odorants for the anterior–posterior differences were not significant for any of these pairs of sniff parameters. This analysis suggests a differential effect of sniff parameters between the medial and lateral parts of the epithelium, but not between the anterior and posterior parts.

Discussion

Contribution of inherent and imposed epithelial properties

The present study was designed to compare responses in a closed preparation with an artificial sniff to a preparation in which the nasal cavity was opened and odorants directly applied to reduce influences dependent on the intact air pathway. These preparations allow comparison of the inherent and imposed properties of the nasal olfactory system mentioned in the Introduction. We used measurements of the response rise time to show that the odorant access varied between these two preparations for the isoamyl acetate, but not for limonene. Measurements of response magnitude showed smaller responses to isoamyl acetate in the lateral recesses of the epithelium of the closed preparation than the opened preparation. This magnitude effect generalized to other polar odorants (carvone and benzaldehyde), but not to the nonpolar hydrocarbons (limonene and benzene).

The electrodes in the opened and closed preparations were placed in positions that were as similar as possible. The results of Vassar *et al.* (Vassar *et al.*, 1993) indicate that the positions we indicate in Figure 1 are in the most medial and most lateral olfactory gene expression zones, suggesting that they might have equivalent intrinsic responses. Our demonstration and the anterior–posterior effects were relatively small in the closed preparation, suggesting that the precise position of the electrodes in either the medial or lateral region was not important. The data of Scott and Brierley

(Scott and Brierley, 1999) support this contention for the dorsomedial recess in the opened preparation. Therefore, it is reasonable to conclude that the differences in responses between the closed and opened preparations reflect some properties related to the passage of air through the intact nasal cavity.

Imposed properties

The model of Mozell and his colleagues (Mozell *et al.*, 1991) provides a likely explanation of our data based on the differential tendencies of odorants to be sorbed by the tissue lining the nose. Mozell (Mozell, 1964, 1970) provided evidence that the retention times on polar gas chromatographic columns could predict the response sizes in the frog epithelium in a way that was dependent on airflow direction. Mozell and Jagodowicz (Mozell and Jagodowicz, 1973) showed that the gas chromatographic retention times also correlated with the times that odorants were retained by frog olfactory epithelium. The odorants that we have used also vary in retention times. Benzaldehyde and carvone have long chromatographic retention times, while benzene and limonene have much shorter retention times (Fuller *et al.*, 1964). We chose to compare the benzene–benzaldehyde and carvone–limonene odorant pairs because of their structural similarity (Figure 5). The behaviors of those odorant pairs are very different and consistent with the chromatographic hypothesis.

Isoamyl acetate was included because previous results in the rat showed a dependence of response on airflow parameters (Ezeh *et al.*, 1995). The isoamyl acetate responses do not easily fit into an explanation based on the literature from the Mozell laboratory (Mozell and Jagodowicz, 1973; Mozell *et al.*, 1991), because its behavior was very similar to that of limonene. However, the rise time data presented here show that isoamyl acetate responses are also strongly influenced by upstream sorption in the rat nasal cavity.

The extension of the chromatographic hypothesis based on results in the frog to the more geometrically complex rodent nasal cavity must be made with caution. The complex geometry of the rodent nose prevents us from applying the model used by Mozell *et al.* (Mozell *et al.*, 1984, 1991) to calculate the number of molecules that pass by the epithelium per unit time, because we are unable to measure the relative flow in the medial and lateral spaces. Therefore, our use of the term 'flow rate' is less formal and precise than their use of the same term. We were also unable to reverse flow direction in the manner that was possible in Mozell *et al.*'s frog experiments (Mozell, 1964; Mozell *et al.*, 1991). However, our comparison of responses in the opened and closed preparations accomplished the same purpose of showing that air flow makes a difference in the response of the epithelium. The same logic was applied by Kent *et al.* (Kent *et al.*, 1996) in showing effects of air flow along the midline of the rat nose.

Our attempts to test for differential effects of flow rate changes on responses to polar and nonpolar odorants similar to those reported by Mozell *et al.* (Mozell *et al.*, 1991) were only partly successful. The responses to isoamyl acetate at the lateral sites increased substantially as flow rate and/or sniff duration were increased, as expected of a polar odorant. Carvone responses showed similar changes at the medial sites. The effects on other odorants of this series were not as impressive. The lateral responses to limonene and benzene were the only cases where the responses in the closed preparation were not significantly smaller than in the opened preparation. This might be attributed to better absorption of these odorants into the epithelium because of a slower flow rate through the lateral spaces or to the other odorants being more effectively removed from the airstream before they reached the receptors. It is likely, for example, that the carvone and benzaldehyde odorants were almost completely sorbed out of the airstream before reaching the lateral recording sites. A wider range of odorants with intermediate physical properties will be necessary to evaluate these ideas.

In summary, in spite of the limitations in our preparations, it is quite clear that the responses were different in the two preparations. Those response differences were particularly evident in the lateral recesses, where the relative stimulation by polar and hydrocarbon odorants was dramatically different between the two preparations. These effects were present across a 10-fold difference in sniff duration and a 3-fold difference in flow rate. In some cases, particularly that of the isoamyl acetate response recorded at lateral sites, the response developed slowly, so that the stimulus duration had a strong effect. A more thorough study of these sniff parameters would require choosing odorants with intermediate ranges of sorption, perhaps near to that of isoamyl acetate, as well as a range of concentrations, flow rates and durations.

Functional implications

These observations strengthen the argument for a relationship between odorant structure and regions of activation in the olfactory epithelium. Although differential partitioning into the mucus based on air flow patterns cannot explain the spatial distribution of responses observed in the opened olfactory epithelium experiments of Scott *et al.* (Scott *et al.*, 1997) or Scott and Brierley (Scott and Brierley, 1999), most of the odorants in those studies that produced maximal responses in the dorsal epithelium are very polar and water-soluble. Both our observations and those of Kent *et al.* (Kent *et al.*, 1996) indicate that the imposed effects of airflow during the sniff act in parallel with the intrinsic properties for these odors. It may be important that the parts of the epithelium facing each other across the lumen of the nasal cavity express the same receptors (Ressler *et al.*, 1993; Vassar *et al.*, 1993) and project to the same parts of the olfactory bulb (Astic *et al.*, 1987; Schoenfeld *et al.*, 1994). This is probably because they are influenced the same

way by the airflow. It is possible that the receptor expression zones and the shape of the nasal cavity may have co-evolved to maximize these effects. Because the sum of the two effects is quite potent, it is likely that this information may be of real use in sensory coding.

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