Spatially Organized Response Zones in Rat Olfactory Epithelium

JOHN W. SCOTT, DONNA E. SHANNON, JEFF CHARPENTIER, LISA M. DAVIS, AND CRAIG KAPLAN
Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322-3030

Scott, John W., Donna E. Shannon, Jeff Charpentier, Lisa M. Davis, and Craig Kaplan. Spatially organized response zones in rat olfactory epithelium. J. Neurophysiol. 77: 1950–1962, 1997. Electroolfactogram recordings were made with a four-electrode assembly from the olfactory epithelium overlying the endoturbinate bones facing the nasal septum. In this study we tested whether odors of different chemical structures produce maximal responses along longitudinally oriented regions following the olfactory receptor gene expression zones described in the literature. The distribution of responses along the dorsal-to-ventral direction of this epithelium (i.e., across the expression zones) was tested in two types of experiments. In one, four electrodes were fixed along the dorsal-to-ventral axis of one turbinate bone. In the other, four electrodes were placed in corresponding positions on four turbinate bones and moved together up toward the top of the bone. These experiments compared the odorants limonene and α-terpinene, which are simple hydrocarbons, with carvone and menthone, which differ from the hydrocarbons by the presence of ketone groups. All responses were standardized to an amyl acetate or ethyl butyrate standard. The responses to limonene and α-terpinene were often larger for the ventral electrodes. The responses to carvone and menthone were largest for the dorsal electrodes. Intermediate electrodes gave responses that were intermediate in amplitude for these odors. The possibility that direction of air flow caused the observed response distributions was directly tested in experiments with odor nozzles placed in two positions. The relatively larger dorsal responses to carvone and relatively larger ventral responses to limonone were present despite odor nozzle position. We conclude that the responses to this set of odors vary systematically in a fashion parallel to the four gene expression zones. The odorant property that governs this response distribution may be related to the presence of oxygen-containing functional groups. Certain odors evoked larger responses at the intermediate electrode sites than at other sites. Cineole was the best example of this effect. This observation shows that not all oxygen-containing functional groups produce the same effect. Although we cannot exclude other possible mechanisms, these three response gradients may be produced by the four receptor expression zones described for many of the putative olfactory receptor genes. Therefore many of the receptors in each zone may share common properties. It remains to be determined whether this zonal input is significant in central odor processing. However, the correlation of odor chemical properties with the structure of receptor molecules in each zone may provide significant leads to structure-function relationships in vertebrate olfaction.

INTRODUCTION

Recent developments have inspired us to reinvestigate the question of distribution of sensitivity to odors on the olfactory epithelium. The particular inspiration for this investigation was the observation that many of the recently discovered olfactory receptor genes (Buck and Axel 1991) are expressed in longitudinal, anterior-posterior-oriented expression zones (Nef et al. 1992; Ressler et al. 1993; Strotmann et al. 1992, 1994; Vassar et al. 1993). Subsequent papers (Buck 1996; Fülle et al. 1995; Ressler et al. 1994; Sullivan et al. 1996) explicitly mention four expression zones, although Strotmann et al. (1994) described only three zones. These zones do not have perfectly sharp boundaries, and there are a small number of genes that are not expressed in this longitudinal pattern (cf. Strotmann et al. 1994). Furthermore, the zonal expression maps are based on study of a relatively small number of genes. Nevertheless, the pattern is sufficiently striking that it is useful to ask whether some odor responses may be distributed in a similar pattern. Such a result would be an important clue to the receptor functions associated with the different genes, which to date have only been directly studied in one published report (Raming et al. 1993).

The question of differential sensitivity in the olfactory epithelium has a long history, and it has largely been studied with the electroolfactogram (EOG), a recording of mass responses of many receptor cells (Gesteland 1964; Getchell 1974; Ottoson 1956). It has long been known that there is a reliable topography of odor responses on the epithelium of the salamander nose (Kauer and Moulton 1974; Mozell et al. 1987), although that topography has been questioned for the frog (Kent and Mozell 1992). Recent EOG (MacKay-Sim and Kesteven 1994) and voltage-sensitive dye (Kent et al. 1996; Youngentob et al. 1995) recordings of the rat olfactory epithelium have shown that there is also a differential distribution of odor response and that the distribution is consistent across animals. However, these investigations of the rat epithelium did not find response distributions that corresponded to the receptor gene expression zones.

This laboratory has recently reported strong correlations of odorant properties with EOG recording positions in the rat olfactory epithelium (Ezeh et al. 1995; Scott et al. 1996). We observed that most odorants with certain oxygen-containing functional groups (especially ketones and aldehydes) tend to produce larger responses in the dorsomedial recess of the epithelium, whereas odors without these functional groups tend to produce larger responses in the ventral and lateral parts of the epithelium, near the base of the turbinate bones. These two regions occupy different gene expression zones. Recordings along the base of the endoturbinates further suggested a correspondence with the gene expression zones. From these observations, we hypothesized that simple hydrocarbons and ketone forms of the same compound would evoke different distributions of response. The relative amplitudes of these responses should vary with recordings from different epithelial regions and would follow a pattern like that of the gene expression zones.

We approached the comparison with expression zones by looking for a longitudinal organization of responses along
the midline of the rat nose, an area where the in situ hybridization of gene probes shows a clear zonal pattern along the medial wall of the endoturbinate bones (Ressler et al. 1993; Vassar et al. 1993). Although those descriptions were made for neonatal rats and mice, they are also present in 21-day-old rats (R. Vassar and R. Axel, personal communication). We adopted a physiological preparation similar to that employed by Youngentob et al. (1995) for their voltage-sensitive dye studies and by Mackay-Sim and Kesteven (1994) for their EOG study. We used terpene odorants, particularly contrasting limonene and α-terpine, which do not have oxygen-containing functional groups, with carvone and menthone, both ketones. We chose these odorants because they are popular olfactory stimuli, because they are relatively rigid molecules, and because a related compound, cineole, evoked an interesting pattern of response in preliminary experiments.

We used four EOG electrodes to allow simultaneous comparison of different sites. The orientations of these electrodes were changed in several experiments to test the hypothesis that odors of different chemical structures would produce maximal responses in longitudinally oriented regions following the olfactory receptor gene expression zones described in the literature. To test the possibility that our response distributions were produced by differential sorption, we have directly compared two air flow paths from opposite directions in some experiments. Some of this work has appeared in abstract form (Scott et al. 1995).

METHODS

Male Sprague-Dawley rats (375–475 g) were anesthetized with ketamine (87 mg/kg), xylazine (13 mg/kg), and butorphanol (0.3 mg/kg). A single tracheal cannula was inserted for respiration. To give the widest exposure of the medial wall of the left endoturbinates, the right eye, skin over the nasal cavity, and zygomatic arch were removed. A large window of bone over the anterior part of the orbital surface, up to and including the base of the zygomatic arch, was removed. When the bone was thin enough to remove, a cotton pad soaked in Ringer solution was placed over the surgical site and the animal was killed. The head was elevated slightly and the animal was allowed to sit for a period of 20–30 min to allow blood flow to stop. In some later animals, those of experiment 1B, the animals were killed before the beginning of surgery. The use of freshly killed animals reduced bleeding and was necessary for maximum exposure of the olfactory epithelium. Previous results show that the responses in this preparation were nearly identical to those in live animals (Scott et al. 1996). Several published EOG (Edwards et al. 1988; Wang et al. 1993) and voltage-sensitive dye (Kent et al. 1996; Youngentob et al. 1995) experiments have used a similar technique with freshly killed animals.

After exposure of the nasal septum, a glass tube (8 mm diam) was positioned 2 cm from the septum, and a constant flow of warmed, humidified air (500 ml/min) was established. This tube was placed along the lower edge of the epithelium at an angle ~30° from the plane of the epithelium. In subsequent descriptions, this tube will be called the odor stimulus nozzle. (In 1 experiment, the position of this nozzle was directly manipulated.) The septum was removed and the septal epithelial tissue overlying the turbinate mucosa was carefully retracted by suction. The entire region from endoturbinate II to endoturbinate IV was exposed from the dorsal margin at the cribiform plate to the ventral margin at the respiratory epithelium (Fig. 1). At the end of each experiment, photographs of the preparation were made through the dissecting microscope with the recording electrodes in place. The same alignment of the microscope was used for each photograph. Blood vessels and other marks were used to locate the tip of the recording electrode under higher power. The tips were marked on the photograph.

Recordings were made with four glass micropipettes filled with Ringer solution, broken to a resistance of 0–5 MΩ. The leads from these electrodes were connected to a four-channel AC coupled amplifier (low-frequency time constant 0.1 Hz). An indifferent electrode was placed on the frontal bone overlying the left olfactory bulb. The outputs were displayed on an oscilloscope and also fed by an A-D converter (digitized at a rate of 53 Hz) to a computer that plotted the traces and computed the peak negative current relative to the baseline just before the stimulus. This peak voltage was printed by each plot and was stored in a computer file for later statistical analysis. The plots were inspected during data collection and before analysis for quality control.

Odor concentrations were generated by forcing air with a syringe pump through the head space in a vial containing the test odor. The four odor vials were connected by Teflon tubing to ports in the odor stimulus nozzle. The choice of the four vials and the rate of the pump were controlled by a BASIC language program. This program was controlled by a standard file sequence of nine sets of traces: a standard stimulus, a set of the three test stimuli, another standard, another set of three test stimuli, and one blank. This sequence is referred to as a “trial” in the description of data analysis. Changing to another set of odors only required changing the tube connections. Most of the odors tested were terpene compounds (Fig. 1B). Each odor response was standardized to the immediately preceding response at the same electrode for the standard odor in the following fashion. The response was divided by the standard response and then multiplied by the average response to the standard odor for all four electrodes. This procedure was adopted to remove variations produced by damage from the electrode tip or differential drying of the tissue. Amyl acetate and ethyl butyrate evoked large responses throughout the olfactory epithelium, but these were usually larger in the ventral part (data not shown).

Odors were presented at a single concentration (a dilution of \(1.8 \times 10^{-1}\)). This represents the fraction of air saturated with odorant in the total volume of air passing the epithelium. The use of a single concentration allowed us to get repeated samples of several odor stimuli for statistical analysis. Recording time was limited because responses steadily declined in voltage over time. This decline is in part due to drying of the tissue, despite our attempts to keep it well humidified. We justified the use of a single high concentration by results in our previous experiments (Scott et al. 1996). There we found that odor producing larger responses in either of two regions of the epithelium did so over the whole concentration range for which they produced measurable responses.

Recording electrodes were set up in three configurations that were aligned to test response distributions relative to the expression zones for the putative receptor genes (cf. Fig. 1). For experiment 1, the four electrodes were oriented along the anterior surface of endoturbinate IV or endoturbinate II* (Fig. 2). Multiple stimulus series were presented with this one arrangement. Electrode 1 was always the dorsal electrode. This orientation was expected to span across most of the receptor gene expression zones. Published information about the expression zones is for neonatal animals. However, R. Vassar and R. Axel have shown (personal communication) very similar arrangements in rats up to 21 days old. For experiment 2, the recording electrodes were maintained in the same position on endoturbinate IV, but the position of the odor stimulus nozzle was varied to test the effect of air flow direction on the response distributions. For experiment 3, we repositioned the recording electrodes to test the responses along the dorsal border of endoturbinate IV (Fig. 5), a position where the expression zones curve into a...
different orientation than that seen on the other turbinate bones. For experiment 4, the four electrodes were oriented along the longitudinal axis of the olfactory epithelium with electrode 1 on endoturbinate IV, electrode 2 on endoturbinate III, electrode 3 on endoturbinate II', and electrode 4 on endoturbinate II (Fig. 6). In each animal we successively tested a set of seven such longitudinally arranged sites at intervals of ~0.5 mm across the dorsal-to-ventral extent of the four endoturbinate bones. Each set of electrode positions was marked on the photograph of the preparation for later analysis.

For experiments 1–3, data were subjected to analysis of variance. In these analyses, the main variables were odor, electrode position, rats, and trial number. The ‘rats’ variable served to control variance introduced by differences in drying or electrode placement between animals. The ‘trial number’ variable removed the variance associated with a small but steady decline in response over the recording session. Because each trial contained two presentations of each odor, we were able to estimate within-trial variances. This procedure gives more realistic estimates of the error bars in the figures. If data from single odor stimulus were excluded because of artifact or equipment failure, then all data from that trial were excluded. To be conservative, we reported all statistical results without the use of the trial number variable to avoid artificially inflating significance values. The odor-by-position interaction was the important result and indicated whether two or more odors differed in the distribution of response magnitude (peak voltage) that they evoked across the electrode array. After finding a significant odor-by-position interaction, we tested the response amplitudes to each odor. This was done in a position-by-rats analysis. The Scheffe procedure was used and, to be conservative, we looked for probability levels of $P < 0.0001$ to report a level of $P < 0.01$. To simplify the reporting, we compared electrode positions 1 and 4 for most odors (carvone, limonene, menthone, etc.) where we expected a fairly simple response gradient. In the case of cineole, we tested whether either positions 2 or 3 gave bigger responses than positions 1 or 4.

For experiment 4, the data were analyzed by multiple regression. A standard overlay of the preparation photographs was produced and used to judge the recording positions. These positions were measured along the dorsal-to-ventral extent of the endoturbinate bone and used as the independent variable in the regression. Similar calculations were made with the use of electrode number as an index of the anterior-to-posterior direction. The former measure gave an estimate of the across-zone variability, whereas the latter gave an estimate of within-zone variability.

**RESULTS**

The overall response amplitudes were smaller than the responses recorded by electrodes that penetrated from the lamina propria side of the epithelium in our previous papers.
FIG. 2. Experiment 1A. A: placement of electrodes for experiment 1. These electrodes were placed at approximately equal intervals along the anterior border of endoturbinates IV or II'. B: average curves for 3 odors in 5 animals for the electrode positions along endoturbinate IV. There were 6–21 trials for each odor per animal (average 15). Error bars: 95% confidence intervals from analysis of variance. C: average responses for 3 animals with the use of the odors in A plus menthone and α-terpinene. Also recorded from endoturbinate IV. There were 9–15 trials for each odor per animal (average 13). Note from Fig. 1 that carvone and menthone are similar in structure, whereas limonene and α-terpinene are also similar in structure. Responses in A and B were standardized to ethyl butyrate. D: average responses for 4 animals (8±12 presentations of each odor per animal) for a similar set of recordings from endoturbinate II'. These responses were standardized to amyl acetate. Note that the labeling of zones is schematic for purposes of orientation and is not meant to imply that a particular electrode was always centered in 1 of the expression zones.

(Ezeh et al. 1995; Scott et al. 1996). The responses in the opened preparation were sometimes quite large at the beginning (up to 12 mV), but often declined within first 10 min. This response decline may be due to slight mechanical damage or drying of the mucosa. It occurred in live animals with opened nasal cavities as well as in the freshly killed preparations (data not shown). Responses were maintained with a slow decline for periods >2 h in most cases. We looked at the data for several animals and determined that responses to all odors declined at the same rate (data not shown). Data were collected as long as responses were stable on all electrodes.

**Experiment 1A**

The comparison of carvone, limonene, and cineole was tested in eight animals with the electrodes arrayed along the anterior border of endoturbinate IV, as shown in Fig. 2A. In each of the five cases making up Fig. 2B, the response to carvone was largest at the more dorsal recording sites. The response to limonene was more nearly flat across the electrodes for the average curve. The limonene and carvone curves progressively diverged in four of the five cases. In analysis of variance the odor-by-position interaction was highly significant (P < 0.01) for the total figure and was
highly significant ($P < 0.01$) for the comparison of carvone with limonene. The response to carvone was larger on electrode 1 and on electrode 4 ($P < 0.001$). The difference between the limonene responses on electrodes 1 and 4 was not significant ($P > 0.05$).

Figure 2C illustrates three other cases that also included $\alpha$-terpinene and menthone odors, structurally similar to limonene and carvone, respectively. The difference between the limonene curves for Fig. 2, B and C, illustrates that there was some individual variation. This variation may arise in part from variations in electrode placement, but at this point it is not completely explained. The parallel carvone and menthone distributions and parallel limonene and terpinene distributions were apparent in all individual cases. The differences between carvone and limonene were also apparent in all individual cases. The responses to limonene and terpinene were greater on electrode 4 than electrode 1 ($P < 0.01$). The responses to carvone and menthone were larger on electrode 1 than electrode 4 ($P < 0.01$).

This experiment with carvone, limonene, and cineole was repeated with simultaneous recordings of responses along endoturbinate II' (Fig. 2D). There the response to carvone was larger on electrode 1 than electrode 4 ($P < 0.01$) and the response to limonene was larger on electrode 4 than electrode 1 ($P < 0.01$).

The cineole curves were not parallel to either the limonene or carvone curves and tended to show a pronounced peak at the intermediate electrodes, although the responses at electrodes 1 and 4 were near the amplitude of the limonene response. In Fig. 2, B and C, the odor-by-position interaction for the limonene versus cineole responses was highly significant ($P < 0.01$). For both panels, the responses to cineole on electrodes 2 and 3 were larger than the responses on electrodes 1 and 2 ($P < 0.01$). For Fig. 2D, the responses on electrodes 2 and 3 were larger than on electrode 1, and the response on electrode 3 was larger than on electrode 4 ($P < 0.01$). In Fig. 2D, the response to cineole was larger on electrode 3 than on electrodes 1 and 4.

The data for Fig. 2 were standardized to responses to amyl acetate or ethyl butyrate as described in METHODS. This allowed us to combine data from different animals in which there were slight differences in the overall amplitude of responses that could be evoked from different electrodes, and reduced the variability in the analyses. The same significant effects were seen in analysis of single animals without standardization (data not shown). In all of these comparisons, there were significant odor-by-position-by-rats interactions ($P < 0.001$). This is likely to have arisen from variations in the placement of electrodes across animals and from variation in the shape of the turbinates. This observation emphasizes the fact that we did not have reliable markers for the expression zones.

**Experiment 1B**

Because cineole produced distinctly different responses from the other odors, we looked for other related odors that might produce peak responses in the intermediate regions (i.e., at electrodes 2 or 3 with the orientation of Fig. 2A). Because cineole is bicyclic, we tried three other bicyclic compounds shown in Fig. 1. Figure 3 shows a comparison of these odors with the odors used in Fig. 2B for three animals. Pinene, a relatively simple compound, evoked a response distribution similar to that of limonene, although not as steep. This distribution had significant odor-by-electrode interactions with the fenchone (a ketone) and 7-oxabicyclo[3.2.1]octan-2-one (an oxepoxide somewhat similar in structure to cineole) responses. All of the odor-by-position interactions in Fig. 3, A and B, are significant at $P < 0.001$.

**Experiment 2**

Two stimulus nozzle positions were compared (Fig. 4). One nozzle was at the same angle as in experiment 1. This was called the ventral position. In some cases, the second stimulus tube was placed at the dorsal extremity of the epithelium directly above the midline, so that it was parallel to the midline epithelium. This was called the dorsal position. In other cases, the second stimulus tube was placed anterior to the recording electrodes at an angle of $\sim 30^\circ$ from the anterior-posterior (midline) axis and at $\sim 30^\circ$ from the dorsal-to-ventral axis. This was called the anterior position.

The effect of stimulus nozzle position is shown in Fig. 4, B and C. The stimulus odor was switched between the two stimulus nozzles in the same animal without changing the position of the tubes or electrodes. Although the curves shown in these plots are slightly different for the dorsal versus ventral comparison (Fig. 4B) and for the anterior versus ventral comparison (Fig. 4C), differences between the response distributions are visible in each condition. All the odor-by-position interactions are present in each part of Fig. 4, B and C ($P < 0.0001$), as in Fig. 2, and show the same relative direction. In four-way analysis of variance (odor by electrode position by tube position) there were significant odor-by-electrode interactions ($P < 0.001$), but the odor-by-electrode-by-tube position interactions were not significant ($P > 0.1$). These comparisons argue strongly that the larger response at the ventral electrode to limonene relative to carvone is not an artifact of air flow in a particular direction.

**Experiment 3**

The expression zones described by Vassar et al. (1993) are not entirely straight, but curve upward at the back of turbinate IV. Therefore the response distribution in the back of this region should be different from that tested in Fig. 2. Figure 5 shows a test of this hypothesis. There is a large difference between the limonene and carvone response distributions and a smaller, but significant ($P < 0.001$) difference between the limonene and cineole response distributions. The space along the dorsal border of turbinate IV is substantially smaller than that along the anterior border. As a consequence, the placement of electrodes relative to expression zones is even less certain than in experiment 1. Nevertheless, the general agreement with the findings of experiment 1 is strong.

**Experiment 4**

Although Fig. 2 shows similar distributions across two endoturbinate bones, these recordings were from different animals, and the resulting curves are of slightly different
FIG. 3. Experiment 1B. Comparison of responses to bicyclic and epoxy compounds. A: odor-by-position interaction is significant for the comparisons of limonene responses with either carvone or cineole responses ($P < 0.01$). $B$: interaction is significant for the comparison of pinene responses with either fenchone or 7-oxabicycloheptane responses ($P < 0.01$). Data from 3 rats with 4–6 presentations of each odor per animal ($A$) and from 2–6 presentations of each odor per animal ($B$). Responses were standardized to amyl acetate.

FIG. 4. Experiment 2. Comparison of responses under different conditions of airflow. $A$: odor stimulation and recording setup. The electrodes were placed along endoturbinate IV as in Fig. 2. The electrodes and nozzles remained in the same position during the experiment, but the odor source was switched between the 2 nozzles after each odor in the series had been tested twice. The standard odor source used in all the other figures was the ventral nozzle illustrated in this figure. The anterior odor nozzle was placed rostral to the recording array, as shown at right. This anterior nozzle was at an angle of $\approx 30^\circ$ to the vertical dimension, as represented by the position of the dorsal nozzle, and $\approx 30^\circ$ to the plane of the epithelium, indicated by the dotted arc. $B$: odor source was switched between the ventral and dorsal nozzles in 2 animals. There were 4–12 presentations of each odor per animal per condition (average 7.6). $C$: odor source was switched between the ventral nozzle and an anterior nozzle directly anterior to the electrode array in 2 animals (8 and 6 presentations of each odor per animal per condition). This anterior nozzle was at an angle of $30^\circ$ relative to the sagittal plane. The tips of the nozzles were 2 cm from the tissue. Amyl acetate was the standardizing odor.
The data from experiment 4 also allowed us to compare whether there was substantial response variation along the anterior-to-posterior direction for cineole, carvone, menthone, and limonene oxide. This odor differs from limonene only by replacing the double bond in the ring with an epoxide. The response distribution to this odor was not quite parallel to that for carvone and menthone but had larger dorsal than ventral responses. The regression curves for each odor had similar shapes across each of the four turbinates (Fig. 7A). There were some differences in the response amplitudes; for example, the carvone responses were largest on endoturbinate II, but the slopes were very similar. The regression curves also had very similar slopes across the four animals (Fig. 7B). These similarities justified plotting overall regression curves for the odors tested (Fig. 7C). These overall curves compare very closely with the plots of Fig. 2, B and C. This analysis is strong evidence that the response distributions shown for the anterior border of endoturbinate IV also extend to the similar surfaces of the other endoturbinates.

Figure 7C also includes a plot for limonene oxide. This odor differs from limonene only by replacing the double bond in the ring with an epoxide. The response distribution to this odor was not quite parallel to that for carvone and menthone but had larger dorsal than ventral responses.

The data from experiment 4 also allowed us to compare whether there was substantial response variation along the anterior-to-posterior direction for these terpene odors. Because measuring anterior-to-posterior distance would require estimating the amount of epithelium buried between the exposed surfaces, we simply used the electrode number as an index of position. Table 1 shows the coefficients of determination for polynomial regression tested with four models. These were along the anterior-to-posterior direction, the dorsal-to-ventral direction, and along the product of the two. The final model involved both directions and the product. This table shows strong, significant coefficients of determination \( R^2 \) along the dorsal-to-ventral direction for cineole, carvone, menthone, and limonene oxide. The \( R^2 \) values were weaker for terpinene and limonene, consistent with the rather flat response distribution for limonene in Fig. 7 and some of the other figures. On the other hand, the anterior-to-posterior measurements (electrode number) accounted for <5% of the variance in the measurements. The cross product of the two directional measures gave results that were intermediate to the two directions, although this model was effective for artificially generated data (not shown) in which responses were larger in the anterior dorsal region than elsewhere. A full model in which the two directions of measurement and the cross product were used did not account for more variance than the dorsal-to-ventral model.
DISCUSSION

Response distribution and gene expression zones

The issue addressed in this work is whether there is a distribution of odor sensitivity in the rat olfactory epithelium that is consistent with the longitudinally oriented receptor gene expression zones. For the terpene odors that we tested, there is such a distribution. Carvone responses were larger than limonene responses in the most dorsal part of the epithelium, which followed the shape of the most dorsal of the expression zones. Limonene responses were larger than or equal to carvone responses in the most ventral and posterior part of the epithelium, in a region roughly corresponding to the most ventral expression zone. Cineole responses were always proportionately larger than limonene responses in the intermediate region of the epithelium. The mean response amplitudes illustrated in this figure follow these trends. Those cineole responses were usually larger in the intermediate region than in either the dorsal or ventral regions. Although the highly significant interactions from the analyses of variance do not do more than demonstrate that the distributions are different for different odors, the reproducibility of the mean curves and the regression analysis strongly agree with these descriptions of responses to those three odors.

Thus the midline distributions of adult rat responses to carvone and cineole appear to correspond to the orientation of expression zones as described for neonatal rats (Vassar et al. 1993). The correspondence for limonene is less strong, but it often evokes larger responses in the most ventral two electrode sites than elsewhere. This interpretation seems compelling despite the differences in animal ages and the fact that we do not have direct visualization of the expression zones for these EOG experiments.

We had already shown reliable differences between the carvone and limonene responses for comparisons between the dorsomedial recess of the epithelium and the ventrolateral recesses of the epithelium between the bases of the turbinatel bones (Scott et al. 1996). The dorsomedial recess corresponds to the dorsal zone in Fig. 1 of the present paper, and the ventrolateral recess probably includes the two ventral zones of Fig. 1 (cf. Ressler et al. 1993; Vassar et al. 1993). Because we did not have reliable access to the intermediate regions in the previous experiments, we did not have information to indicate the difference between the cineole and limonene response distributions. Note that the present data agree with that previous report in showing that the cineole response is consistently larger in the ventral part of the epithelium. Taken together, the two EOG studies are a strong
FIG. 7. Combined polynomial regression analysis from 4 animals in experiment 4. A: regression curves and individual points for comparisons across turbinate bones. Data from the 4 animals were pooled in these calculations. Error bars: 95% confidence intervals for the regression line. Limonene and carvone are illustrated because they showed the most and least scatter, respectively, around the regression curves. All regression curves were 2nd order. B: curves for the odors comparing the 4 animals and pooling data from the 4 turbinate bones. One set of points for each odor (of 196 points) was removed before the calculations of A and B, because an anomalous response to the standard odor inflated all of the data values for 1 electrode. C: regression curves for 6 odors from experiment 2. The curves for carvone, limonene, and limonene-oxide are based on 4 animals, whereas the others are based on 3 animals. The standardizing odor was ethyl butyrate.

### TABLE 1. Coefficients of determination

<table>
<thead>
<tr>
<th></th>
<th>Terpinene</th>
<th>Limonene</th>
<th>Cineole</th>
<th>Carvone</th>
<th>Menthone</th>
<th>Limonene Oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>DV</td>
<td>0.37 ± 0.15</td>
<td>0.16 ± 0.09</td>
<td>0.51 ± 0.11</td>
<td>0.74 ± 0.08</td>
<td>0.61 ± 0.10</td>
<td>0.35 ± 0.10</td>
</tr>
<tr>
<td>AP</td>
<td>0.02 ± 0.15</td>
<td>0.04 ± 0.18</td>
<td>0.03 ± 0.15</td>
<td>0.00 ± 0.16</td>
<td>0.00 ± 0.15</td>
<td>0.00 ± 0.12</td>
</tr>
<tr>
<td>DV × dAP</td>
<td>0.15 ± 0.17</td>
<td>0.12 ± 0.09</td>
<td>0.14 ± 0.14</td>
<td>0.46 ± 0.12</td>
<td>0.43 ± 0.12</td>
<td>0.26 ± 0.11</td>
</tr>
<tr>
<td>Full</td>
<td>0.42 ± 0.14</td>
<td>0.22 ± 0.09</td>
<td>0.57 ± 0.10</td>
<td>0.76 ± 0.08</td>
<td>0.61 ± 0.10</td>
<td>0.34 ± 0.10</td>
</tr>
</tbody>
</table>

*R² (mean ± SE) for polynomial regression models including only the dorsal-to-ventral (DV) or anterior-to-posterior (AP) axes and models including both DV and AP axes with cross product. All of the unidirectional models used quadratic equations. The “full” model combined terms for DV, DV², AP, AP², and DV × AP.*
argument for a relationship between the putative gene expression zones and differential sensitivity to at least this set of odors.

The difference between the limonene and carvone (or terpinene and menthone) responses was graded over the parts of the epithelium tested. There were not clear discontinuities that would support radical differences in the binding properties of receptors in adjacent zones for these odors. This was true even in experiment 4, in which individual animals were tested with greater spatial resolution, and the relative amplitudes of limonene and carvone responses changed gradually across the dorsal-to-ventral extent of the turbinate bones. This fact may result from the indistinct borders of receptor gene expression zones (Vassar et al. 1993), although it should also be taken as a caution against a premature conclusion that the response distribution results exclusively from the zonal distribution as described by the available probes. One possibility is that future expression mapping may demonstrate other zones slightly out of register with the ones currently known. Such a family of zones would be easier to reconcile with the gradual transitions suggested in our data.

The systematic gradients of response are surprising given the very large number of receptor genes projected for the rodent and indications that any individual gene is expressed in <1% of the receptor neurons (Buck 1996). These data could be explained by a small number of receptor cells in the dorsal zones being strongly responsive to the ketones (such as carvone) but not responsive to the hydrocarbons (such as limonene). Alternatively, many of the receptors expressed in a zone might have some common properties, so that most cells of the dorsal zone would be more responsive to ketones than to hydrocarbons. We have previously argued for the latter interpretation because strong stimuli like the ones used here would almost completely block anticholinergic activation of the receptor cell population (Ezeh et al. 1995). The final resolution of this question will require extensive sampling of single receptor cells, something that is beyond the scope of this study.

If there are common properties shared by most receptors expressed in a single zone, one would expect some structural similarity of the genes expressed within a zone to correlate with the differential distribution of response magnitudes. Although members of the same receptor gene subfamily are generally located in the same expression zone (Buck 1996), there is no strong sequence similarity of genes expressed in the same zone. Singer and Shepherd (1994) have recently modeled the olfactory receptors, suggesting that the binding pocket for odors lies among the transmembrane helices of the protein in a position analogous to that of the β-adrenergic receptor. Singer and Shepherd (1994) have recently found, in analysis of 21 mouse receptor sequences from Sullivan et al. (1996), that there is a significant correlation between the residues occurring in position 430, which Singer and Shepherd believe to be part of the binding site, and the expression zones. In particular, Singer and Shepherd (personal communication) find a high incidence of histidine in this site in the most dorsal zone but no histidine in this position in other zones. Indeed, histidine 430 has been predicted to interact selectively with oxygen-containing groups (Singer et al. 1995). Assuming that the same principles apply to the rat, this may be a very fruitful area of study.

**Possible generalization to other odors**

Although the strongest data are for the three odors described above, several other related odors had response distributions that were predictable from their structure. Pinene and α-terpinene evoked larger responses ventrally than dorsally. Fenchone and menthone had larger responses dorsally. This supports our previous generalization that terpene ketones evoke their largest responses in the dorsal zones. We had previously extended that generalization to other groups of odorants, including alkanes, cyclic alkanes, and aromatic compounds. We also reported that some other compounds with oxygen-containing functional groups (some aldehydes and some epoxides) evoked larger responses in the dorsal region than the corresponding hydrocarbons, although alcohols were equivocal (Scott et al. 1996). We have not yet extensively tested those here, but some data support the previous generalizations. Limonene oxide was more effective dorsally in experiment 4, with some tendency toward a peak response in the intermediate zone. In the few cases tested, methyl benzoate evoked larger dorsal responses than did toluene, and cyclooctanone evoked larger dorsal responses than cyclooctane (data not shown). These comparisons were similar to those we had previously reported. Therefore we predict that the observations on comparison of the dorsomedial and lateral recesses of our previous paper will extend to the most dorsal and most ventral expression zones wherever they are tested.

The precise property of the odorant that determines the response distribution cannot be specified from these data alone. Imamura et al. (1992) reported distributions of responses in the olfactory bulb that are consistent with these data and suggested that the carbonyl group was an important determining factor in their data. The fact that ketones produce a greater proportion of dorsal over ventral responses than similar aldehydes or esters (Scott et al. 1996) suggests that more than the presence or absence of the carbonyl group is involved. This point is also obvious in our present data, in which all responses were standardized to esters. On the other hand, Scott et al. (1996) also found that aromatic esters and an aldehyde had much more pronounced localization than the aliphatic esters. In this report, the fact that limonene oxide had a greater tendency for dorsal responses also indicates that a more general property is involved. One possibility is the degree of electronegativity of the functional group, as suggested by Sato et al. (1994).

We also showed in a previous paper (Ezeh et al. 1995) that responses to several odors showed little variation along the anterior-to-posterior direction in either the dorsal or lateral parts of the epithelium. The present paper demonstrates that response differences (particularly those between limonene, carvone, and cineole responses) follow the distribution along the medial wall of the endoturbinate bones that has been described for expression of putative olfactory receptor genes in the neonatal rat (Vassar et al. 1993). Our observations are merely correlations with the expression zones, and more data will be necessary to demonstrate a causal relationship. Nevertheless, the correspondence seen here and in our
previous papers (Ezeh et al. 1995; Scott et al. 1996) encourages us to believe that these distributions are related to the expression zones.

A more complicated condition is presented by the distribution of responses to cineole. This distribution was always different from the distributions of either limonene or carvone responses in that it usually had a maximum in intermediate regions of the epithelium. This result was not seen in our previous papers, in which we did not have access to the intermediate regions of the epithelium. This observation makes it clear that the simple presence or absence of oxygen-containing compounds is not the only determinant of response distribution. The fact that cineole is a less polar compound than carvone or menthone may be relevant. This intermediate peak for cineole suggests that the intermediate zones may contain receptors maximally activated by a different property than the properties governing the most dorsal or most ventral responses. We cannot more generally specify optimal stimuli for these intermediate regions. Not all bicyclic compounds produced this response distribution. The compound 7-oxabicycloheptane evoked responses closest to the cineole distribution. The other epoxy compound, limonene oxide, also had a response distribution that was intermediate between that of carvone and menthone on the one hand and cineole on the other hand.

**Other potential explanations of the data**

One area of concern in our previous paper was whether the observed response distributions could be produced by differential sorption of odors onto the epithelium, thus removing them from the air stream and leading to a gradient of stimulus concentration across the epithelium. This phenomenon is well established under certain conditions (Hahn et al. 1994; Kent et al. 1996; Mozell et al. 1987). There are several arguments against this suggestion. At the flow rate and angles used, there may be minimal exposure of the odor in the air stream to the epithelial surface. Differential sorption might account for the difference between carvone, which is highly sorbed, and limonene, which is not highly sorbed, but a more complicated mechanism would have to be invoked for the cineole response distribution. The fact that the odor effects follow the particular course of the putative receptor gene expression zones would require peculiar assumptions about air flow turbulence in the cavity. Visual inspection of the rise times of the carvone and limonene responses (data not shown) does not support a consistent relationship with air flow direction as would be expected from the sorption hypothesis. The strongest statement is the fact that odor-by-position interactions in the same direction were present with three different air flow directions. These considerations lead us to believe that the response distributions reflect a mechanism that is intrinsic to the epithelium rather than one imposed by air flow.

This conclusion does not contradict the demonstrated effect of differential sorption on odor response distributions. That effect can be strong both along the anterior-posterior length of the midline of the rat epithelium (Kent et al. 1996), and in comparisons between the dorsal recess and the spaces between the bases of the turbinate bones (Ezeh et al. 1995). This is in agreement with a number of demonstrations of this effect in the frog (reviewed in Hahn et al. 1994). Sorption is purposely minimized in the present series of experiments, but it is likely to contribute strongly to sensory processing.

Odorant binding proteins (Pevsner et al. 1985) and mechanisms that internalize and break down odorants have been described (Lazard et al. 1991). There is some evidence for spatial segregation of odor binding proteins (Rama Krishna et al. 1995). The action of either of these activities could influence the odor responses observed here if they acted selectively on odorants with particular chemical properties. Until the specifics of the distributions of these materials are better known, we must be guarded in the tentative conclusion that we have observed a property of odor molecules that is bound by receptors of particular expression zones.

**Comparisons with recent literature**

Several recent papers (Kent et al. 1996; Mackay-Sim and Kesteven 1994; Youngentob and Kent 1995; Youngentob et al. 1995) investigated the distribution of odor responses along the midline of the nose and did not observe the strong longitudinal orientation of responses reported here. We believe that this difference from the present report results from the particular odors sampled in each of the studies. We chose to test odorants that we expected to produce response gradients across the zones on the basis of the presence of certain functional groups tested in our previous paper (Scott et al. 1996). We contrasted compounds that differed primarily in this property. With the exception of Figs. 6 and 7, we did not make comparisons along the longitudinal axis of the expected gene expression zones. The other authors were attempting to sample odorant properties more generally. Their odor stimuli differed in several characteristics. Unfortunately, they did not use more than one of the odorants in our series, so direct comparisons with our contrasting responses with limonene and carvone are not possible. It is possible that some of the response gradients that the other authors observed are related to some of the nonzonal distributions reported in the literature (Strotmann et al. 1994).

We have not compared odors that differ in carbon chain length in this series. This is an important variable in determination of response in olfactory bulb mitral cells (Mori and Yoshihara 1995). Our previous recordings with homologous series (Scott et al. 1996) did not explore a sufficiently large range of chain length to test for regional differences with this variable. This would be an important variable to study in the future. It seems possible that one of the reasons that other epithelial recordings have not produced the dorsal-to-ventral response gradients like those we observe is the fact that they have mixed several molecular properties, including chain length and different chemical classes, in their stimulus series (Kent et al. 1996; Mackay-Sim and Kesteven 1994; Youngentob et al. 1995). EOG and voltage-sensitive dyes are appropriate techniques for study of epithelial properties given the fact that all such investigations have reported systematic regional variations. The large numbers of genes expressed in the epithelium and the low probability of finding any individual gene expressed in a particular cell (Buck 1996) mean that principles learned about the classes of odors that are effective in stimulating receptor cells in different
regions of the epithelium may be very useful in selecting stimuli for study of individual cells.

Implications for sensory processing

Finally, we can make a few comments on the relationship of these data to sensory processing. Because we have sampled only a small number of odor classes, many more data are necessary to specify the relationship between gene expression zones and olfactory sensations. The reliable relationship that we observe between odor response and recording position is not strong enough to account for olfactory experience. Carvone, menthone, and limonene oxide have detectably different odors to humans. We are also aware that individual receptor neurons even within a particular region of the epithelium may have different response properties (Sato et al. 1994). Recent results on the projection pattern of olfactory sensory neurons (Ressler et al. 1994; Vassar et al. 1994) agree with neurophysiological (Imamura et al. 1992; Mori and Yoshihara 1995) and other (Guthrie et al. 1993; Lancet et al. 1981) results in arguing for a convergence of axons from sensory neurons with like properties onto small numbers of glomeruli. This convergence is doubtless very important in arranging the sensory information for processing in the olfactory bulb.

The fact that each part of the epithelium projects its axons to a restricted region of the olfactory bulb (Aestic et al. 1987; Jastreboff et al. 1984; Pedersen et al. 1986; Schoenfeld et al. 1994) indicates that the bulb must have an input organization that represents the odor chemistry differences expressed in the epithelium in some systematic way. This is already indicated by recordings from rabbit mitral cells (Mori and Yoshihara 1995). The long mitral cell basal dendrites span a distance that may correspond to a substantial portion of the input from one of the expression zones (Orona et al. 1984). The intrabulbar association described by Schoenfeld et al. (1985) clearly allows for interactions of part of the bulb receiving inputs from different expression zones. It would be important to contrast the function of these systems in analyzing olfactory information.

There is not yet enough information to propose the chemical principle that determines whether an odor will produce its largest response on one part of the epithelium. So far we have tested only hydrocarbon odors and those with functional groups limited to oxygen atoms. The polarity of the molecule is one property that is altered in most of these compounds. Many more tests will be necessary to determine whether this single property is enough to account for the three response gradients that we have observed.

The authors thank F. H. Schmidt for aid with instrumentation and data analysis.

This work was supported by National Institute of Deafness and Other Communications Disorders Grant DC-00113.

Address reprint requests to J. W. Scott.

Received 27 June 1996; accepted in final form 10 December 1996.

REFERENCES


