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An electroolfactogram study of odor response patterns from the mouse olfactory epithelium with reference to receptor zones and odor sorptiveness

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Coppola DM, Waggener CT, Radwani SM, Brooks DA. An electroolfactogram study of odor response patterns from the mouse olfactory epithelium with reference to receptor zones and odor sorptiveness. *J Neurophysiol* 109: 2179–2191, 2013. First published January 23, 2013; doi:10.1152/jn.00769.2012.—Olfactory sensory neuron (OSN) responses to odors, measured at the population level, tend to be spatially heterogeneous in the vertebrates that have been studied. These response patterns vary between odors but are similar across subjects for a given stimulus. However, few species have been studied making functional interpretation of these patterns problematic. One proximate explanation for the spatial heterogeneity of odor responses comes from evidence that olfactory receptor (OR) genes in rodents are expressed in OSN populations that are spatially restricted to a few zones in the olfactory epithelium (OE). A long-standing functional explanation for response anisotropy in the OE posits that it is the signature of a supplementary mechanism for quality coding, based on the sorptive properties of odor molecules. These theories are difficult to assess because most mapping studies have utilized few odors, provided little replication, or involved but a single species (rat). In fact, to our knowledge, a detailed olfactory response "map" has not been reported for mouse, the species used in most studies of gene localization. Here we report the results of a study of mouse OE response patterns using the electroolfactogram (EOG). We focused on the medial aspect of olfactory turbinates that are accessible in the midsagittal section. This limited approach still allowed us to test predictions derived from the zonal distribution of OSN types and the sorption hypothesis. In 3 separate experiments, 290 mice were used to record EOGs from a set of standard locations along each of 4 endoturbinates utilizing 11 different odors resulting in over 4,400 separate recordings. Our results confirmed a marked spatial heterogeneity in odor responses that varied with odor, as seen in other species. However, no discontinuities were found in the odor-specific response patterns across the OE as might have been predicted given the existence of classical receptor zones nor did we find clear support for the hypothesis that OE response patterns, presumably a reflection of OSN distribution, have been shaped through natural selection by the relative sorptive properties of odors. We propose that receptor zones may be an epiphenomenon of a contingent evolutionary process. In this formulation, constraints on developmental programs for distributing OSN classes within the OE may be minimally related to the odor ligands of specific class members. Further, we propose that odor sorptiveness, which appears to be correlated with the inherent response patterns in the OE of larger species, may be of minimal effect in mice owing to scaling issues.

olfactory-sensory neurons; sorption; maps; coding; chemotopy

SENSORY EPITHELIA CONTAIN arrays of receptor cells that transform specific types of stimuli into neural signals that, excluding illusions, are the necessary prerequisites for perceptions. The spatial distribution of receptors on these surfaces is often dramatically nonuniform to gain efficiency in stimulus capture. For example, the density of small-receptive-field somatosensory receptors in the human skin is greatest on the fingertips and lowest on the back, partly explaining the difference in two-point discrimination between these regions (Vallbo and Johansson 1984). The cone receptor density in the human retina is more than an order of magnitude greater in the fovea than in the periphery affording substantially greater visual acuity in the center of view (Osterberg 1935). In these two examples, the functional significance of the spatial distribution of receptors is apparent: to maximize the collection of useful information from the environment, the hand used to manipulate objects, and the fovea functioning as the center of gaze in an eye repositionable through saccades (e.g., Andrews and Coppola 1999).

In contrast, the olfactory system has no obvious spatial dimension, save a decreasing concentration gradient with distance from a stimulus source. What need then could there be for a nonuniform distribution of receptor cells in olfactory sensory surfaces? More than a half century ago, Adrian (1942, 1950) proposed that olfaction, like the other senses, may have a spatial component. He posited that the physical properties of odors, such as volatility and water solubility, determine a spatial pattern of stimulation across the OE that could aid in odor discrimination. Despite the many discoveries and technical advances of the intervening decades, the idea that olfaction has a spatial dimension remains an active area of research (Rojas-Líbano and Kay 2012; Schoenfeld and Cleland 2005, 2006; Scott 2006).

Beginning with the classic work of Mozell (1964, 1966) on frogs, it has been repeatedly shown in terrestrial vertebrates that inhaled odors distribute themselves across the OE in a pattern that depends on their sorptiveness, suggesting an analogy between the initial phases of olfaction and gas chromatography (reviewed by Scott 2006). That these "imposed" patterns of odor separation have functional significance has been most clearly demonstrated by electrophysiological recordings and other response measures from the OE (Moulton 1976). First in the frog (Mozell 1970) and later in the rat (Kent et al. 1996), it was shown that response patterns in the OE are correlated with the patterns of odor separation based on sorptiveness. In addition, in a heroic series of studies utilizing the electroolfactogram (EOG) to measure the generator potential of ensembles of ORs in the rat, it was shown that the OE possesses an "inherent" pattern of odor sensitivity, which compliments the imposed patterns observed by Mozell and colleagues (Moulton 1976; Scott et al. 1996, 1997, 2000; Scott and Brierley 1999). Thus, when delivered directly on the exposed OE, highly water-soluble odors such as benzaldehyde caused

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greater responses in upstream areas while hydrophobic odors, such as hexane, caused greater responses in downstream areas (Scott 2006; see Fig. 2). To date, Scott and colleagues have tested several dozen odorants from a variety of different chemical classes, most of which produced results that are in substantial agreement with the sorption hypothesis, i.e., that spatial separation of odors in the OE due to a chromatographic effect (imposed pattern) is correlated with the inherent pattern of odor responses in the OE (Schoenfeld and Cleland 2005, 2006; Scott 2006).

Of course the most transformative findings related to the role of the OE in odor discrimination came with the discovery of the family of G protein-coupled olfactory receptors (Buck and Axel 1991). Molecular and histochemical techniques that allowed the visualization of an individual OR gene's expression among the ~1,000 genes present in rodents revealed that each receptor is expressed, with rare exceptions, within a single longitudinal zone, originally thought to be four in number (Ressler et al. 1993; Vassar et al. 1993). Although the function, if any, of these zones remains a mystery, EOG recordings in the rat reveal that the largest variability in OE responses occurs across zonal boundaries implicating them in the regional specificity that might underlie sorption-based odor discrimination (Scott et al. 1996, 1997, 2000; Scott and Brierley 1999).

Despite the sorption hypothesis' long history, only a small number of species have had OE responses mapped. In some previous studies, only a few odors and recording locations were used providing only a fragmentary picture of regional odor responses (e.g., Edwards et al. 1988; Norlin et al. 2005; Troitskaia 1988). The rat is the only mammal that has been studied in detail, a regrettable situation given that most molecular studies are still carried out in the mouse (e.g., Bader et al. 2010; Bozza et al. 2009; Miyamichi et al. 2005). Another motivation for mapping olfactory responses in the mouse is that its nasal morphology and airflow patterns are quite similar (though not identical) to those in the rat (Jiang et al. 2010). Thus, if airflow patterns, the driving force (i.e., mobile phase) of chromotographic odor separation, have been a determining factor through the action of natural selection in regional specificity of the OE, the mouse would be expected prima facie to resemble the rat with respect to predictions of the sorption hypothesis.

The work to be described provides, to our knowledge, the first detailed study of odor response patterns in the OE of the mouse. Because of the complex geometry of olfactory turbinates, only the medial aspect of endoturbinates II, II', III, and IV were studied, a constraint also true of the majority of studies in the rat. However, this limited approach still allowed sampling across all the receptor zones and included a substantial portion of the air path through the olfactory ethmoturbinates. Thus the results allowed us to evaluate the impact of zonal distribution of receptors on the OE response patterns and to test predictions of the sorption hypothesis.

Consistent with previous studies in amphibians and mammals, we found pronounced spatial heterogeneity of EOG responses in the mouse OE. In agreement with studies in the rat, we also found that the majority of the variability in responses occurred perpendicular to the predicted boundaries of receptor zones, suggesting that the observed response pattern is due to OR distribution. However, we did not find evidence of sharp zonal boundaries, despite closely spaced sampling in some instances. Finally, we found little evidence to support the sorption hypothesis, despite the inclusion of extremely hydrophobic and hydrophilic odors in our stimulus set. We suggest that the smaller size of the mouse OE together with the mouse's higher metabolic rate and thus higher respiration rate may make odor sorption unimportant in this species. However, on theoretical grounds, we also argue that odor sorption may not be a practical dimension of quality coding in any species.

MATERIALS AND METHODS

Animals. All animal procedures were approved by Randolph-Macon College's Internal Animal Care and Use Committee and conformed to the National Institutes of Health *Guidelines for Care and Use of Laboratory Animals*.

A group of 290 female, outbred, CD-1, adult mice (Charles River Laboratories, Wilmington, MA) were used in three separate experiments. Only one gender was used to minimize size differences between subjects; however, the large age range (35–180 days) added to the variability in subject size in the first and largest experiment. In the *experiments 2* and *3*, the age range was limited to 40–55 days to minimize size differences. Mice were housed four per cage (191 × 292 × 127 mm) in a standard animal care facility with ad libitum access to food and water under a reversed 12:12-h light cycle. All recordings were done during the animals' subjective night phase.

Surgical preparation. Immediately before electrophysiological recording mice were killed with a lethal dose of Nembutal (70 mg/kg ip), decapitated, and had the skin removed from their skulls. Previous EOG studies in rats have established that recordings from freshly killed animals are virtually indistinguishable from those obtained from live anesthetized animals for periods up to several hours (Scott et al. 1996). A disposable microtome blade was used to separate the left and right sides of the skull along the midsagittal plane. For the majority of recordings, only the left hemisection was used since preliminary studies confirmed no detectable differences between sides. To expose the recording area, the right nasal septum and overlying tissue were resected to reveal the medial aspect of endoturbinates II, II', III, and IV. Immediately after surgery, the preparations were maintained in a humidified chamber until recordings were completed. Room temperature was kept below 20°C to preserve the viability of the preparation.

Recording setup. Recordings took place in a Faraday cage covered with plastic sheeting. The chamber was suffused with the output of an ultrasonic humidifier and a forced air humidifier that, besides increasing humidity, maintained positive pressure in the recording chamber. Humidity readings taken in the vicinity of the preparation during recording sessions typically approached 98%. Once the preparation was immobilized in the recording chamber, the recording electrode was positioned at predetermined locations on the medial surface of the endoturbinates under microscopic guidance using a three-axis manipulator. For all recordings, the indifferent electrode was placed by hand on the frontal bone at its intersection with the cribriform plate and was held in place with a magnetic clamp. The recording electrodes consisted of Ag/AgCl wires inside glass capillaries filled with 0.05% agar in 0.1 M PBS that had been pulled to an ~50-μm tip diameter. The indifferent electrodes consisted of Ag/AgCl wire inside a 500-μl pipette tip filled as above. Electrodes were connected to the inputs of an Iso-DAM8A DC Amplifier (World Precision Instruments, Sarasota, FL) with low-pass filtering at 10 Hz the output of which was sampled at 20 Hz by a PowerLab/8SP (AD Instruments, Colorado Springs, CO) for A/D conversion, display, and recording. The dependent variable in all three experiments was the EOG maximum amplitude, measured manually for each trace with the use of the LabChart software within PowerLab.

Stimuli were carried to the mucosal surface in a 0.5-s pulse of air (700 ml/min) from the headspace above a 10-ml mixture of odorant dissolved in mineral oil or distilled water contained in a 25-ml vial. The carrier gas was charcoal-filtered room-air that was humidified before entering the stimulus apparatus. A custom unit consisting of computer, software, interface, and olfactometer (Knosys, Lutz, FL) controlled stimulus duration and timing. For most recordings, odor

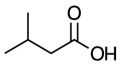
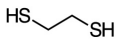
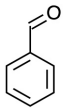
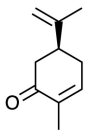
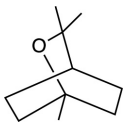
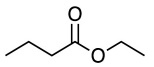
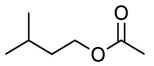
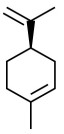
type and concentration were set by manually switching the reservoir vial that was in line with the odor port. For some recordings, a custom-made computer-controlled, eight-channel olfactometer was used to switch the odor type for a particular recording. The odor delivery port was a 3-cm long, 3.5-mm diameter glass tube connected by a 3-cm long Teflon tube to the odor reservoir vial. A three-axis micromanipulator was used to position the odor port 10 mm from the point at which the recording electrode made contact with the OE. A rigid guide-hair was used to maintain a consistent standoff distance and angle of the odor port in relation to the surface of the OE.

Several hours of postmortem viability have been reported in similarly prepared rats that were kept at 8–10° lower ambient temperatures than attempted in this study (Scott and Brierly 1999). A series of preliminary studies (data not shown) established that our preparations stayed viable in excess of an hour with little or no decline in EOG responses. Thus all recording sessions were limited to 60 min in duration.

Stimuli. The stimuli consisted of 11 different odors (Table 1) all single molecules at or near the highest purities (>97 to >99%) commercially available (Sigma-Aldrich). The panel of odors was constructed so as to include chemicals that had been used previously in olfactory physiological and psychophysical studies and spanned a wide range of odor qualia and physical properties, particularly those related to sorptiveness (Schoenfeld and Cleland 2005, 2006).

Concentrations were prepared as the vol/vol dilution of odor in the diluent. Odors were dissolved in mineral oil except those with the three lowest Henry's Law coefficients in which water was the diluent. The actual concentration of gas-phase odor reaching the mucosal surface was unknown. In *experiments 1* and *3*, only a single concentration of odorant (0.1%) was used. Both our preliminary studies (data

Table 1. *List of odorants used in this study along with some of their relevant chemical and physical properties*

Odorant	Class	Quality	Molecular weight, g/mol	Vapor Pressure, Torr (25°C)	K_{Henry}, atm-m^3/mol	Structure
Isovaleric acid	Carboxylic acid	Putrid vomit	102.13	0.554	9.96E-07	
Pyridine	Aromatic heterocyclic	Putrid	79.10	9.99	6.00E-06	
Ethane dithiol	Thiol	Rotten cabbage	94.2	5.61	2.03E-05	
Benzaldehyde	Aromatic aldehyde	Almond aromatic	106.12	0.974	2.81E-05	
Nonanol	Fatty alcohol	Fresh floral	144.26	0.041	5.48E-05	
S-carvone	Terpenoid	Aniseed aromatic	150.22	0.066	7.73E-05	
Eucalyptol	Cyclic ether	Camphorous aromatic	154.25	1.65	1.31E-04	
Ethyl butyrate	Ester	Fruit pineapple	116.16	12.79	3.15E-04	
Isoamyl acetate	Ester	Banana etherous	130.18	5.68	5.35E-04	
R-limonene	Terpenoid	Citrus lemon	136.23	1.54	3.80E-01	
Nonane	Alkane hydrocarbon	Ethereal	128.26	4.63	4.77E+00	

Note ordering according to Henry's Law coefficient (K_{Henry}).

not shown) and published work on rats (Scott and Brierley 1999) established that the spatial patterns of odor responses across the OE are fairly constant with varying concentrations except near the threshold or ceiling of the EOG response. The 0.1% concentration used in most experiments represents approximately the upper third quartile of EOG response magnitudes for isoamyl acetate, the odor from our panel that triggered the strongest response at most loci on the OE.

To verify that the use of a single concentration was justified, two concentrations of each of two odorants, nonane and benzaldehyde, were used in *experiment 2*. The two concentrations for each odor where chosen independently based on the results of preliminary studies (not shown) so as to invoke a roughly similar percent difference in EOG responses from each concentration. However, as in *experiments 1* and *2*, the exact concentrations delivered at the OE were unknown and immaterial to the goals of the study.

Experimental designs. A well-known challenge of using the EOG to make fine quantitative distinctions in OE responses is its imprecision caused by variability in electrode placement, stimulus delivery, and the temporal instability of the preparation both at a particular recording location and across the entire preparation (Scott and Scott-Johnson 2002). Most investigators have used the technique of normalizing responses in some way, often to a standard odorant such as amyl acetate (e.g., Mackay-Sim and Kesteven 1994; Scott and Brierley 1999). However, our preliminary studies revealed a strong anterior-posterior gradient in response to isoamyl acetate that could create a substantial bias depending on the normalization procedure. Also, it was desirable to avoid the repeated stimulation of each recording location with a single odor that is required for normalization. Therefore, randomization and extensive replication were used to overcome the inherent imprecision of the EOG (Waggener and Coppola 2007). We also decided against using multiple simultaneous recording locations, as has often been done in previous experiments (e.g., Mustaparta 1971; Scott and Brieley 1999). In a series of preliminary studies, it was determined that the placement of the odor port contributed far more to the variability of the EOG response than electrode repositioning. In any event, the close quarters inherent in recording EOGs from the mouse compared with larger species that have been studied, such as the rat, tiger salamander, and frog, make impractical recording from multiple loci simultaneously. Therefore, a single electrode was repeatedly repositioned as was the stimulation port with care taken to place the latter at the same distance and angle to the recording electrode (35°) using the guide hair mentioned previously. In *experiment 1*, recording locations along the dorsal edge of each turbinate were based on relative measurements given the large differences in animal size. For example, in each subject, turbinate II was measured along its rostrocaudal extent and divided into five equivalent segments that provided six recording locations including the termini of each segment. Given their shorter rostrocaudal extents, turbinate II' and III were divided into three segments with four recording locations, and turbinate IV, the smallest, was divided in half giving three recording locations. Recording locations along a given turbinate were never >2.5-mm apart; however, these absolute measurements were not otherwise evaluated systematically.

For a given recording session the order of turbinate, recording location and odorant delivery were chosen randomly without replacement. Once the electrode was positioned at a recording location, responses were recorded at 50-s intervals for all five odors and the blank without disturbing the recording electrode. We set the minimum sample size at 20 subjects per location with the average sample size of 25 (range 20 to 41). Data were analyzed using JMP 8.0.2 (SAS Institute, Cary, NC) excluding data from the blank stimulus to avoid biasing the results. A separate ANOVA was used to analyze the results from each turbinate using subject as a random effect within a model with odor, location, and their interaction as fixed effects [restricted or residual maximum likelihood (REML) method within JMP]. Also in the model was a test for the nonlinear component of location, which was modeled as a continuous variable. Given that there was a random effect in the model, "the variance does not partition in the usual way, nor do the degrees of freedom attribute in the usual way, for REML-based estimates" (*JMP Statistics and Graphics Guide*, 2nd ed., SAS Institute). Thus only the F ratios and probabilities are reported. However, in the graphic representation of these data we used the more conservative plot of 95% confidence intervals (CIs) of the mean response at each recording location based on the t-distribution (Prism 5; GraphPad Software, La Jolla, CA). The means and CIs allow the results from any two recording locations to be readily compared but do not protect the overall (experiment wise) statistical error rate.

Olfactory receptor zones lie roughly perpendicular to the rostrocaudal axis of the endoturbinates examined in this study. Thus the recording locations along a turbinate sampled different zones, although exact zone boundaries were unknown. Conversely, recording locations within the same ordinal position on different turbinates lie roughly parallel to the receptor zones and therefore do not tend to cross zone boundaries. Given this anatomical arrangement, there should have been more variability in responses as recording location moved along a turbinate (rostrocaudal) than among turbinates (dorsoventral) if zones indeed convey any specificity in odor response. To test this prediction, the coefficient of variation (CV) was calculate from the mean responses to each odor within turbinates and among turbinates at each ordinal position. Mean responses for position five and six on turbinate II could not be used for the among-turbinate calculation because they had not counterpart on the other turbinates. The four CVs for within turbinate responses (turbinates II, II', III, and IV) were compared with the four CVs for among turbinate responses (positions 1–4) using the Mann-Whitney test. While this strategy of assessing response variability was ad hoc, it was very conservative given that it had few assumptions and a low type-one error rate.

In *experiment 2*, the responses of turbinates II and IV were probed using two odors, nonane and benzaldehyde, at two concentrations each. Recording locations were determined by absolute measurements given that animal size variation was limited by using similarly aged animals, as note above. For turbinate II recordings, locations were chosen at 1-mm intervals for a total of 10. For the shorter turbinate IV, recording locations were chosen at 0.5-mm intervals for a total of eight. As in *experiment 1*, the order of recording from turbinate-location combinations for each animal was determined by random selection without replacement. Once the electrode was placed at a location, both odors at each concentration were used as stimuli as well as a blank with the order being determined by a random process without replacement. Not all recording locations could be visited in every animal given the predetermined time limit (60 min). However, every turbinate-location combination was replicated in at least 20 subjects (mean 28.6). The statistical analysis and graphical representation proceeded as in *experiment 1* with separate ANOVAs for each turbinate. Subject was used as a random effect in the REML model with odor and location as fixed effects.

For *experiment 3*, four previously untested odors served as stimuli: ethyl butyrate, nonanol, pyridine, and ethane dithiol. All odors were delivered as the headspace above 0.1% vol/vol solution. Responses were recorded at the same locations on turbinate II as in *experiment 2*. As in the previous experiments, the order of recording location and stimulus were randomized. However, unlike *experiments 1* and *2*, recordings were replicated twice with repositioning of the electrode for each location in each of eight subjects. The statistical analysis proceeded as in *experiment 1* with ANOVA analysis on the mean of the two replicates using animal as a random effect in the REML model (within JMP) with odor, location, and their interaction as fixed effects.

RESULTS

Experiment 1. The goal of *experiment 1* was to determine if there was spatial heterogeneity of OE responses that was odor

specific and consistent across subjects as seen in other species (e.g., Mozell 1964; Mustaparta 1971; Mackay-Sim and Kesteven 1994; Scott and Brieley 1999; see Moulton 1976). A diverse odor set was chosen in an attempt to detect any chemotopy that might exist in the mouse OE since ours was the first detailed study of its kind.

A photomicrograph of the mouse ethmoturbinates from the midsaggital view after the septum had been partially excised illustrates the approximate recording locations for *experiment 1* (Fig. 1*A*). The natural yellow coloration of the tissue in this area is coextensive with OE. A diagram of the preparation, shown as Fig. 1*B*, was created to aid in the comparison of results of this experiment with those of *experiments 2* and *3*, as well as published work on other species.

Consistent with the results of previous studies (e.g., Mustaparta 1971; Mackay-Sim and Kesteven 1994; Scott and Brieley 1999) marked differences in response magnitude were recorded at different rostrocaudal positions along the turbinates that were similar across animals for a particular odor. EOG traces from two animals responding to isoamyl acetate at recording locations along turbinate II' illustrate both the response variability at a given location and the consistency in the pattern across locations (Fig. 1*C*).

The compiled results involving 2,109 separate EOG recordings in 175 subjects confirmed that all five odorants produced different absolute magnitudes of EOG response, with different spatial patterns both along a given turbinate and among turbinates (Fig. 2). For turbinate II, odor ($F = 222.5$; $P < 0.0001$),

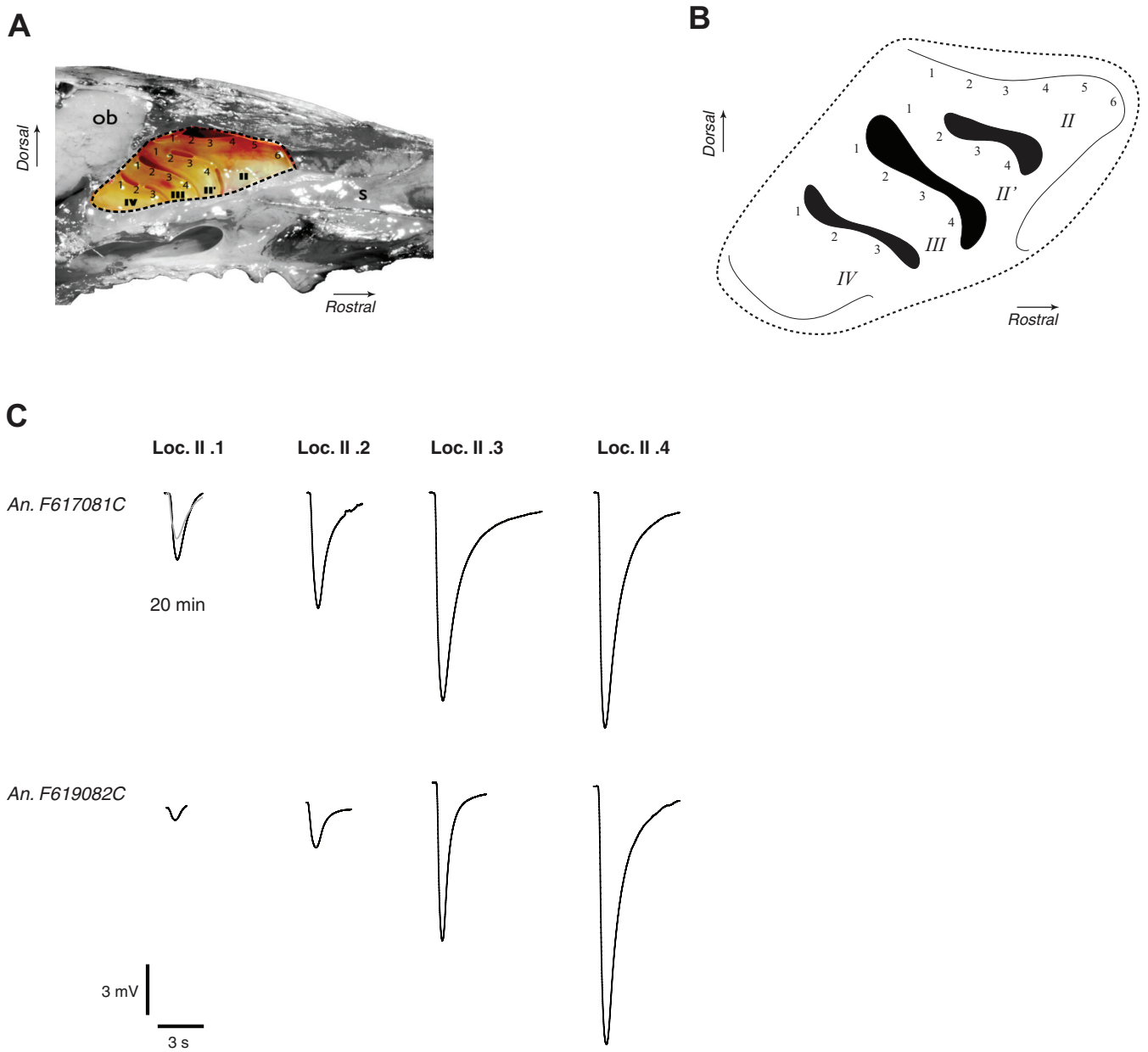


Fig. 1. *A*: photomicrograph of midsaggital view of mouse head in hemisection. Dashed line encloses exposed ethmoturbinates labeled with roman numerals and numbered recording locations. Note: natural yellow color, which is coextensive with olfactory epithelium. *B*: diagram of turbinate recording locations for *experiment 1* used for comparison with other experiments. *C*: raw electroolfactogram (EOG) traces at 4 locations on turbinate II' from 2 subjects in response to isoamyl acetate. Gray line at location II'.1 shows replicate EOG taken following electrode repositioning 20 min after the first recording.

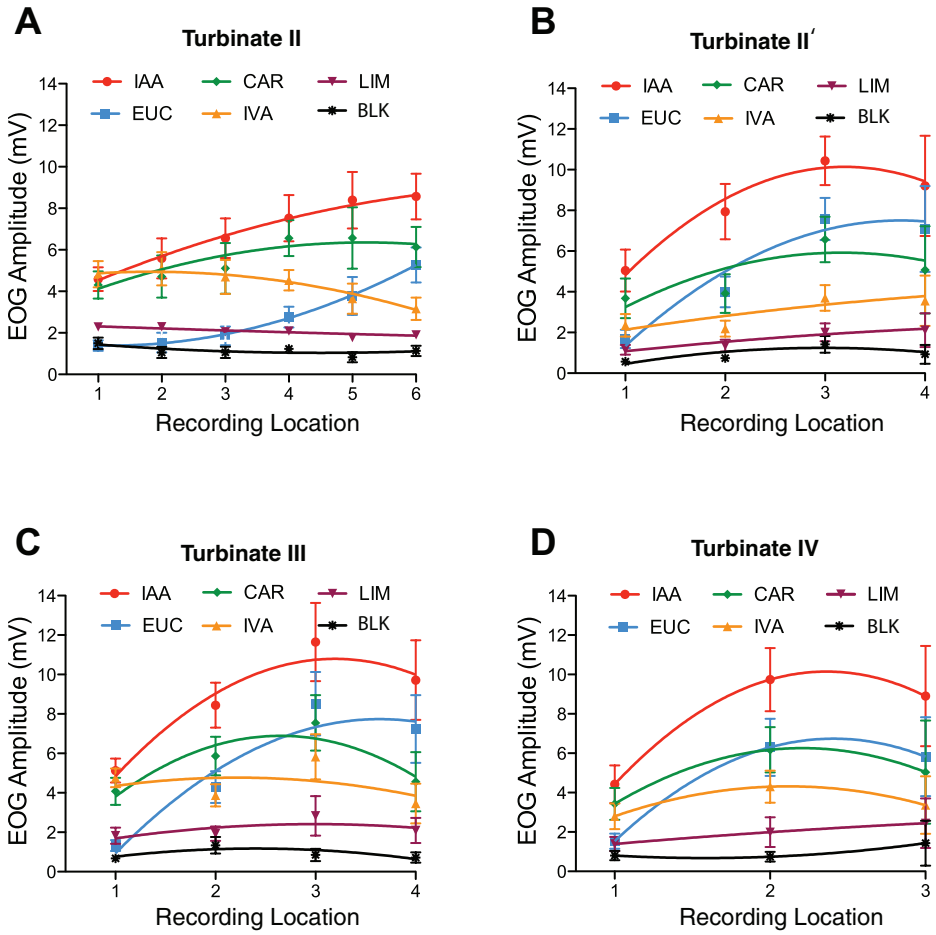


Fig. 2. Means ± 95% confidence intervals (CIs) of EOG amplitudes (absolute values) at each recording location for each turbinate in *experiment 1*. *A*: results for turbinate II. *B*: results for turbinate II'. *C*: results for turbinate III. *D*: results for turbinate IV. For all graphs lines are 2nd-order polynomial fits of the data. IAA, isoamyl acetate; EUC, eucalyptol (1,8-cineole); CAR, D-carvone; IVA, isovaleric acid; LIM, D-limonene; BLK, blank. Note: mean responses for all the blanks were significantly different from zero (see text for statistical results and sample sizes).

recording location ($F = 92.3$; $P < 0.0001$), and their interaction ($F = 53.1$; $P < 0.0001$) were significant terms in the ANOVA; however, the nonlinear term for location was not significant. For turbinate II', odor ($F = 77.8$; $P < 0.0001$), recording location ($F = 100.1$; $P < 0.0001$), and their interaction ($F = 9.6$; $P < 0.0001$) were significant terms in the ANOVA as was the nonlinear component for location ($F = 15.8$; $P < 0.0001$). For turbinate III, odor ($F = 89.6$; $P < 0.0001$), recording location ($F = 69.5$; $P < 0.0001$), and their interaction ($F = 28.8$; $P < 0.0001$) were significant terms in the ANOVA as was the nonlinear component for location ($F = 50.2$; $P < 0.0001$). Finally, for turbinate IV, odor ($F = 86.3$; $P < 0.0001$), recording location ($F = 7.1$; $P < 0.001$), their interaction ($F = 19.1$; $P < 0.0001$), and the nonlinear component for location ($F = 6.6$; $P < 0.01$) were all highly statistically significant factors in the ANOVA.

Examination of the patterns of mean responses provides no evidence of abrupt changes from location to location and most of the data could be well fit by a quadratic polynomial. The majority of odor responses in turbinates II', III, and IV tended to be greatest near the middle of each structures' rostrocaudal extent, but this tendency had exceptions. Notably, the blanks, while giving the lowest responses, had significantly nonzero values presumably due to the fact that the OSNs are known to respond to mechanical force, in this case from the airstream used to deliver the stimulus (Grosmaitre et al. 2007). Indeed, in a series of preliminary studies with thoroughly ethanol-cleaned and oven-dried blank stimulus cartridges, nonzero EOG responses were always observed suggesting that these results are not due to contamination.

The results of our analysis of CVs, in which we compared the variability of mean responses among turbinates vs. within turbinates, are shown in Fig. 3. Only isovaleric acid gave more variable mean responses among turbinates than within turbinates, a difference that was not statistically significant. For the

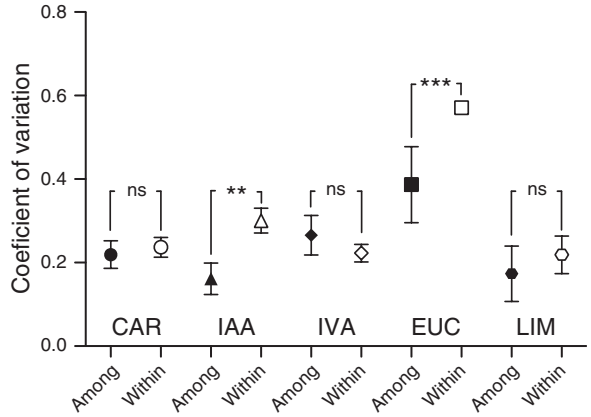


Fig. 3. Mean coefficients of variation (CV) are plotted for the mean EOG responses among turbinates at the same dorsoventral location (all location-1 mean response for example) and within turbinates (along turbinate II mean responses for example) for each odor. Mean CV for a given odorant were compared using the Mann-Whitney test. Ns, nonsignificant. $^{**}P < 0.02$ and $^{***}P < 0.01$. Odor abbreviations are as in Fig. 2.

remaining odors, in which there was more variability within than among turbinates, only the results for isoamyl acetate and eucalyptol reached statistical significance (Fig. 3; Mann-Whitney, $n = 4$, one-tailed $P < 0.02$ and $P < 0.01$, respectively). Also, the omnibus test of all five odors, comparing the CVs among vs. between turbinates, was not significant (Wilcoxon for matched-pairs, $n = 5$, one-tailed $P > 0.09$).

As noted previously, two lines of investigation suggest possible explanations for any stereotyped pattern of responses observed in the OE. The first posits that odor sorptiveness causes differential deposition of odorants along the respiratory pathway (a chromatographic effect) that has, through natural selection, produced an "inherent pattern" of OSN distribution to take advantage of these physical constraints (reviewed by Moulton 1976; Schoenfeld and Cleland 2005, 2006). Recent studies using computational fluid dynamics provide insights into the aerodynamics of the rodent nasal cavity (Jiang et al. 2010; Kimbell et al. 1997; Zhao et al. 2006). Inspired air travels rapidly through a dorsomedial path along turbinate II and then turns rostroventrally to exit the nasopharnx after passing along turbinates II', III, and IV (Fig. 4*A*).

A potentially related proximate explanation for the spatial pattern of responses from the OE emerges from the discovery that the distribution of OSNs expressing a given OR is constrained to a limited number of zones (Fig. 4*B*; Ressler et al. 1993; Vassar et al. 1993). Thus, if a zone defines a family of OSNs with a particular chemical range, any complete chemotopic map of the OE would be expected to evidence a zonal signature (Scott 2006).

To assess these possible explanations for the response patterns illustrated in Fig. 2, the data were replotted based on the position of recording locations along the airstream for turbinates II (most upstream) and turbinate IV (most downstream). The strategy is shown in Fig. 4*C* for two odors, carvone and limonene, which have dramatically different water solubility and have been used previously in studies of odor sorption (reviewed by Scott 2006). Consistent with the sorption theory and previous modeling and empirical research, carvone had a declining trend of response as the recording location moved from upstream to downstream in the odor path ($R^2 = 0.78$; $P < 0.002$). In contrast, limonene, a highly hydrophobic molecule did not have a significant trend in response along the airstream ($R^2 = 0.06$; $P > 0.5$). To further probe the relationship between sorptiveness and inherent OE response patterns the slope of the responses along the airstream were plotted against the Henry's Law constant (HLC) for the five odorants in *experiment 1* (Fig. 4*D*). These constants were estimated with the US Environmental Protection Agency's EPISuite using the "group method" in HENRYWIN for 25°C. Low values represent high sorptiveness a characteristic that takes into account a combination of physical features including water solubility, polarity, and volatility. To be consistent with the sorption theory molecules with low HLCs should have tended, like carvone, to have negative slope (decreasing responses moving down the airstream path) and molecules with high HLCs should have tended to have positive slopes (increasing responses moving down the airstream path). However, of the five odors used in *experiment 1*, only carvone had a significant gradient of response across the airstream. Indeed, isovaleric acid, a substantially more sorptive molecule than carvone, had a flat response profile that was not significantly different from zero, and eucalyptol, with a HLC similar to carvone, had a positive slope, although this was not statistically significant.

Experiment 2. The goal of *experiment 2* was to further evaluate whether odor sorption and zonal distribution could explain EOG response patterns. Two potential shortcomings of *experiment 1* were the use of a single odor concentration and the relative infrequency of spatial sampling (~2- to 2.5-mm intervals). In this experiment, two odor concentrations were used and EOGs were recorded at 1-mm intervals along turbinate II

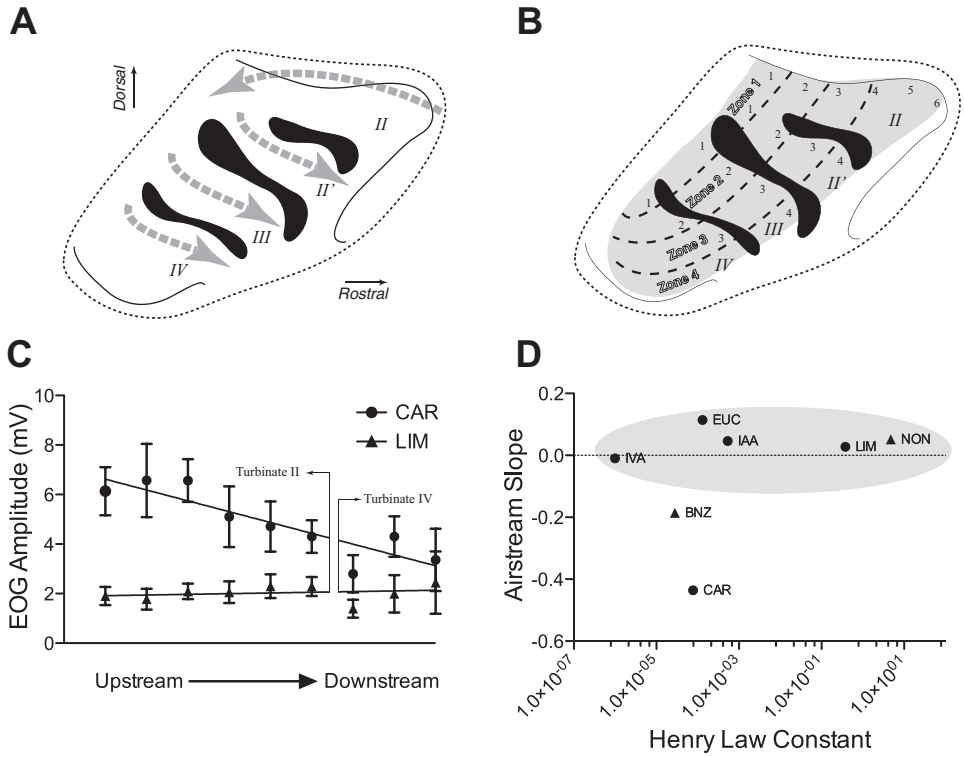


Fig. 4. *A*: predicted airflow pattern during inhalation through area of EOG recording (Zhao et al. 2006; Jiang et al. 2010). Note: air entrance rostrodorsally and exit ventrally. *B*: diagramatic representation of four canonical receptor zones through area of EOG recording (Ressler et al. 1993; Vassar et al. 1993; see also Scott 2006). *C*: data from Fig. 2 for turbinate II and IV are replotted relative to the position of each recording location along the air path (*A*). Mean responses across the air path for the water-soluble odorant carvone, which has a significant negative slope and the relatively insoluble odorant limonene, which does not have a significant slope (see text). *D*: slopes of air path mean responses (as in *C*) plotted against Henry's Law constants for each odor used in *experiments 1* and *2*. Odorants within the gray oval do not have significant slopes (see text).

and 0.5-mm intervals along turbinate IV (Fig. 5*A*). Stimuli were benzaldehyde and nonane that have HLCs differing by more than five log units (Table 1). Benzaldehyde, an aromatic aldehyde, is a water-soluble, low-volatility odorant that has been used in previous sorption research (reviewed by Scott 2006). Nonane is a volatile alkane hydrocarbon, which is estimated to be 15,000 times less soluble in water than benzaldehyde (EPISuite).

Consistent with *experiment 1*, the results of *experiment 2* provide little evidence for abrupt changes in odor responses at either spatial scale of sampling (Fig. 5, *B–E*). In fact, the nearest-neighbor recording locations had overlapping CIs with but one exception (cf. locations 6 and 7; Fig. 5*C*). The ANOVA results for turbinate II responses were significant, not surprisingly, for the main effect of concentration, both for benzaldehyde ($F = 33.2$; $P < 0.0001$) and nonane ($F = 28.4$; $P < 0.0001$). Despite some spatial heterogeneity in the results, location was not a significant factor for either benzaldehyde ($F = 0.88$; $P > 0.34$) or nonane ($F = 0.18$; $P > 0.65$) and there was not a significant linear trend for either odor at either concentration. The ANOVA results for turbinate IV were significant, as expected for the concentration main effect, both for benzaldehyde ($F = 38.3$; $P < 0.0001$) and nonane ($F = 97.7$; $P < 0.0001$). In contrast to turbinate II, location was a significant term in the ANOVA on turbinate IV responses, for both benzaldehyde ($F = 151.1$; $P < 0.0001$) and nonane ($F = 4.2$; $P < 0.05$). In addition, for benzaldehyde there was a significant concentration and location interaction ($F = 24.5$; $P < 0.001$).

To make it easier to examine the changes in OE responses across the entire airstream, the data were replotted, as done in *experiment 1*, with airstream position as the plot's abscissa and EOG amplitude as the plot's ordinate (Fig. 5*F*; see Fig. 1*A*). Graphed in this way the results did not show a trend for either the lower ($R^2 = 0.04$; $P > 0.43$) or the higher ($R^2 = 0.04$; $P > 0.43$) concentration of nonane. However, benzaldehyde responses, at both concentrations, showed a significantly decreasing trend (lower $R^2 = 0.24$; $P < 0.04$; higher $R^2 = 0.31$; $P < 0.02$). The slopes of the trend lines for both odors are included in Fig. 4*D*.

Experiment 3. The results of *experiments 1* and *2* establish that the patterns of responses, as one moves from rostral to caudal recording locations on turbinates II', III, and IV, tended to be similar for a given odor and were greatest near the middle of the turbinate for most odors. However, turbinate II, with the largest area of OE, had response patterns with variable rostrocaudal trends across odorants. For example, in *experiment 1*, limonene and isovaleric acid had significant positive rostrocaudal trends in response ($R^2 = 0.80$; $P < 0.02$ and $R^2 = 0.83$; $P < 0.01$, respectively) while isoamyl acetate ($R^2 = 0.97$; $P < 0.0003$), carvone ($R^2 = 0.77$; $P < 0.02$), and eucalyptol ($R^2 = 0.90$; $P < 0.003$) showed negative trends (Fig. 2). In *experiment 2*, by contrast, nonane and benzaldehyde stimulation did not result in significant linear response trends across turbinate II at either of two concentrations (Fig. 5).

Given these results the goal of *experiment 3* was to further explore rostrocaudal response trends in turbinate II, across 10 recording locations at 1-mm intervals (Fig. 5*A*) using 4 additional odors. Ethylbutyrate was chosen because it is structurally similar to isoamyl acetate, the odorant that evinced a response trend with the greatest slope in turbinate II (Fig. 2*A*).

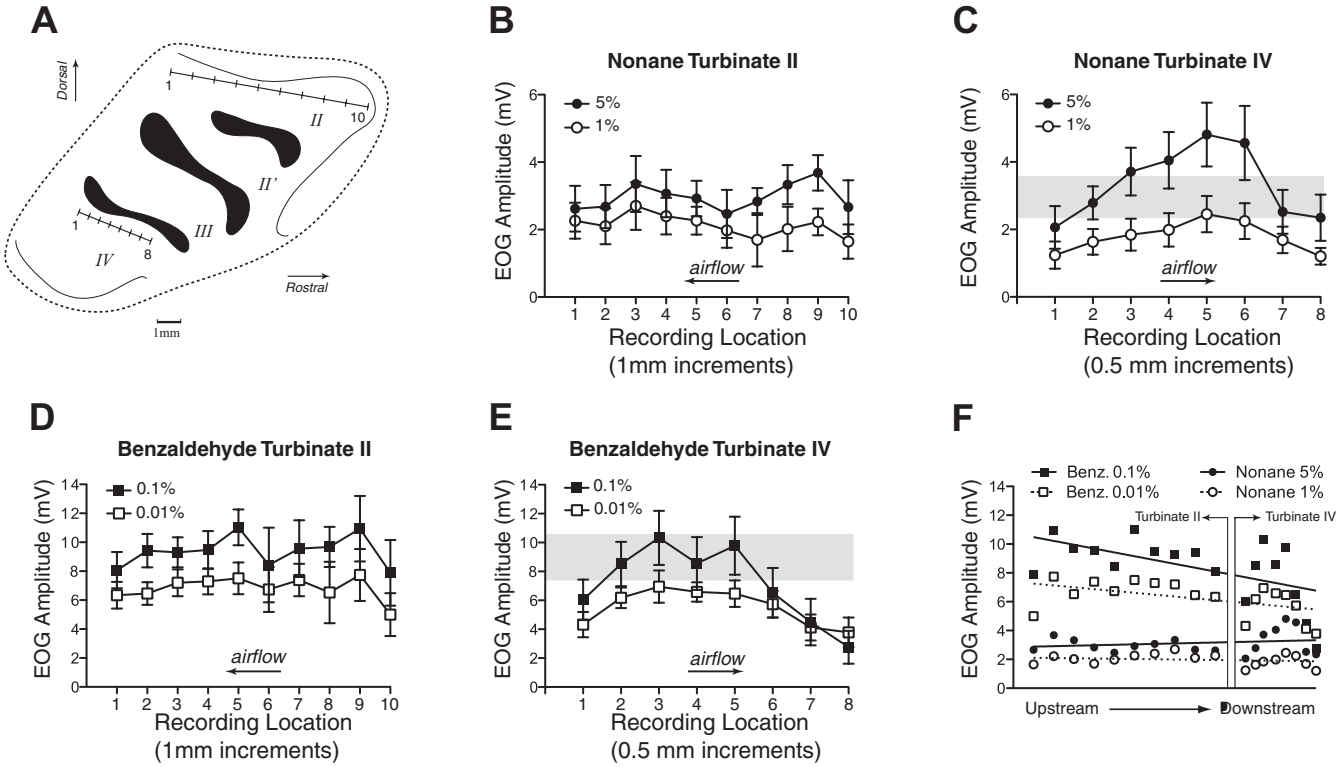


Fig. 5. *A*: recording locations used in *experiments 2* and *3* were determined by absolute measurements. Note: hash marks for turbinate II are at 1-mm intervals and those for turbinate IV are at 0.5-mm intervals. *B*: means ± 95% CIs of EOG amplitudes (absolute values) at each recording location on turbinate II for 2 concentrations of nonane. *C*: tubinate IV results for nonane. *D*: turbinate II responses for 2 concentrations of benzaldehyde. *E*: turbinate IV results for benzaldehyde. Gray bars in *C* and *E* represent grand means ± 95% CIs of the adjacent turbinate II results for comparison.

Similarly, nonanol was chosen because it is identical to nonane save for the alcohol functional group. Pyridine and ethane dithiol were chosen because they are rather hydrophilic molecules, with "putrid" or "rotten" odor qualities that have been more rarely used in olfactory research. In addition, it has been suggested that turbinate II, which resides in the dorsomedial part of the ethmoid turbinates, may have some specialization for odors with aversive qualities (Kobayakawa et al. 2007).

Since there were only eight (or fewer) replicates in this experiment for each odor, average responses for each of the recording locations could be plotted separately by subject (Fig. 6). For a given odorant, considerable subject-to-subject variation was in evidence both within a given recording location and in the pattern of responses across recording locations. Nevertheless, for the omnibus ANOVA, odor ($F = 36.2$; $P < 0.0001$), location ($F = 5.49$; $P < 0.0001$), and their interaction ($F = 1.60$; $P < 0.03$) were significant effects. Since trends in response were our main interest in this experiment the rest of the discussion of results will emphasize regression analysis. Of the four odorants only ethyl butyrate showed a significant linear trend in responses ($R^2 = 0.24$; $P < 0.001$) with a positive slope as the recording location was moved from caudal to rostral locations. The positive slope for ethyl butyrate is consistent with the results for isoamyl acetate, a very similar molecule, however, the magnitudes of the slopes were substantially different (ethyl butyrate = +0.45; isoamyl acetate = +0.84). Nonanol, with an inverted U-shaped response profile that was fit best by a second order polynomial, did not differ markedly from the nonane profile from *experiment 2* (Fig. 6). Pyridine and ethane dithiol, two of the most water soluble odorants in the study, failed to show any discernable trend in response across recording locations. Although absolute response levels were not of particular interest in this study, it is notable that these two putrid odorants tended to invoke weaker responses than ethyl butyrate, which human subjects often describe as having "fruity" or "pineapple"quality. The greater average magnitude in response to ethyl butyrate compared with ethane dithiol was statistically significant using the post hoc test Tukey's honestly significant difference, while this difference only reached statistical significance for recording locations 7–10 with pyridine (*t*-test, uncorrected for multiple comparisons).

DISCUSSION

Our results, including >4,400 separate EOG recordings using 11 different odorants, provide the first detailed study of regional differences in odor responses in the mouse OE. This study doubles the number of mammals that have been examined, since only the rat had been investigated previously at this level of detail. Thus the results provide the first opportunity to address whether OE response patterns are conserved across two rodent species separated by >20 million years of evolution (www.timetree.org) but that otherwise have roughly similar niches as cosmopolitan, muroid omnivores that frequently exist as human commensals.

At the most basic level of analysis our results are consistent with previous mapping studies in amphibians (Mozell 1964; Mustaparta 1971) and rats (Mackay-Sim and Kesteven 1994; Scott et al. 1996, 1997, 2000; Scott and Brierley 1999) in that they demonstrate significant spatial heterogeneity in OE responses that is odor specific and consistent across individuals. In the most extreme instance two recording locations along turbinate III had more than a sixfold difference in average response for eucalyptol (Fig. 2*C*). Similar, if less dramatic, regional differences were observed for several of the odorants including isoamyl acetate, carvone, and isovaleric acid (Fig. 2).

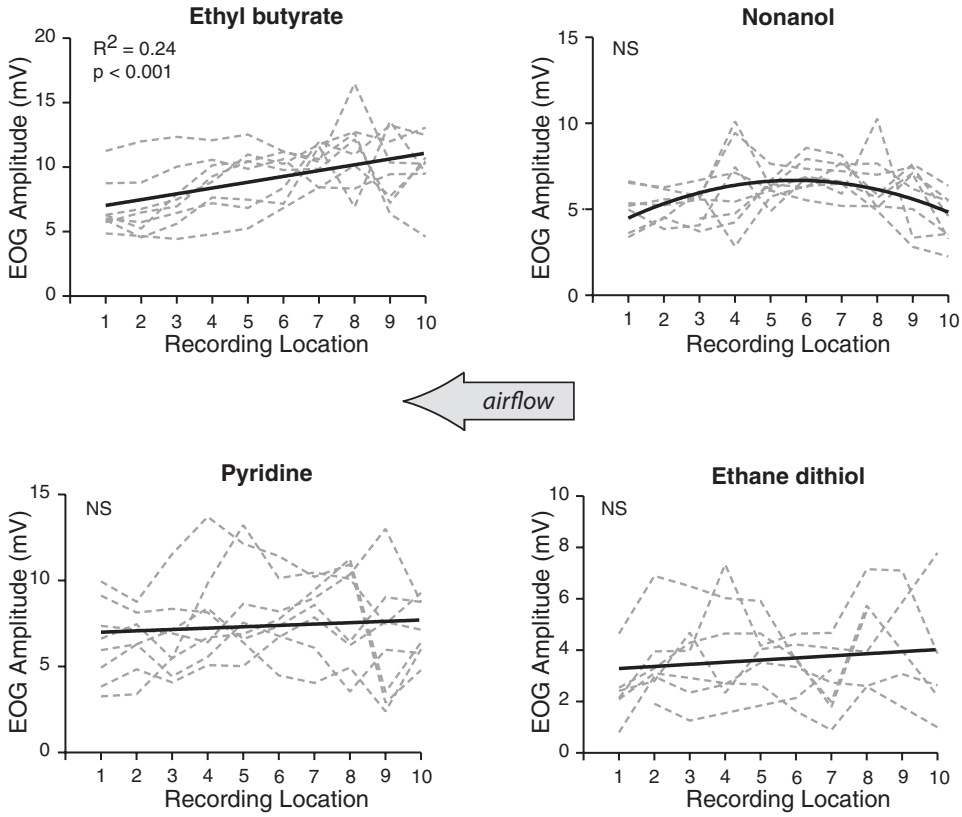


Fig. 6. EOG amplitudes (absolute values) at each recording location on turbinate II from *experiment 3*. Dashed lines show results for each animal individually. Solid lines are the best linear or 2nd-order polynomial fit. Note: the response profile for ethyl butyrate had the only significant slope.

Consistent with detailed studies in the rat (Scott et al. 1996, 1997, 2000; Scott and Brierley 1999), OE response variability was greater along than among turbinates for four out of five odors in *experiment 1*; however, these differences only reached statistical significance for two odorants (Fig. 3). Thus our results provide some evidence that OSN ligand specificity may underlie the observed regional response pattern given that advancing the recording electrode along the longitudinal axis of turbinates crosses predicted OR zonal boundaries, while moving the electrode among turbinates, at the same longitudinal position, does not (see Fig. 4*B*). Although changes in thickness of the musosa, regional distribution of chaperone molecules, enzymes, or other olfactory modulators might explain spatial patterns of response, none of these factors are known to vary across the OE in the observed pattern (Scott 2006).

Locating zonal boundaries. The original work on OR zones in rodents suggested that there may be a few, perhaps four, nonoverlapping zones within which a given OR gene's expression was widely and randomly distributed among OSNs (Ressler et al. 1993; Vassar et al. 1993; Sullivan et al. 1996). However, later investigators established that some OR genes are expressed in atypical patches (Strotmann et al. 1994) and that OR gene expression zones may be broadly overlapping rather than having discrete boundaries (Iwema et al. 2004; Miyamichi et al. 2005). To the extent that our EOG recordings reflected OR gene spatial distribution, none of the three experiments in this study support the existence of sharp zonal boundaries. The response profiles for the five odorants in *experiment 1* were well fit by a linear equation or quadratic polynomial (Fig. 2). However, given that recording locations in this experiment were determined with relative measurements and that the spatial sampling frequency was comparatively low, zonal boundaries may have been missed. *Experiment 2* addressed both of these shortcomings by using absolute measurements in similarly sized mice and by sampling more densely. Even with 0.5-mm sampling intervals we could not detect any abrupt changes in response magnitudes in adjacent recording locations using two concentrations of nonane or benzaldehyde. Indeed, mean responses from all 10 of the adjacent recordings locations on turbinate II (1-mm sampling) had overlapping CIs for both odorants at both concentrations. For turbinate IV, all but one pair of the eight adjacent recording locations (0.5-mm sampling) had average responses with overlapping CIs but even this one abrupt change was only observed with the higher concentration of nonane (Fig. 5). *Experiment 3*, while only providing data on turbinate II, also failed to show any spatial discontinuity in responses that was uniform across animals. These findings are consistent with the observations that OR zones have broadly overlapping spatial distributions (Iwema et al. 2004; Miyamichi et al. 2005). Of course, it remains possible that boundaries exist between zones but that the broad tuning of the ORs within those zones prevented us from detecting them with our methods. However, at least provisionally, we conclude that OR spatial distribution underlies the spatial heterogeneity of responses in the OE but that this distribution is broadly overlapping, consistent with the smooth gradients in response profiles that we observed.

Sorption theory. The sorption theory makes clear predictions about the inherent (in contrast to imposed) spatial pattern of OE responses: ORs sensitive to highly water soluble odorants should be localized to OSNs positioned closer to the incoming airstream (henceforth referred to as "upstream") while ORs sensitive to weakly water soluble odorants should be positioned further back ("downstream"; Schoenfeld and Cleland 2005, 2006; Scott 2006). These predictions are clearly met for a host of odor molecules in the rat OE, although detailed data on other mammals are lacking (reviewed by Scott 2006). In an expansive study involving EOG recordings on the medial aspect of turbinate IV, it was shown that water-soluble odorants including benzaldehyde and carvone had declining response profiles with distance along the turbinate in the downstream direction (Scott et al. 2000). By contrast, weakly soluble odorants such as limonene and octane showed increasing response profiles downstream (Scott et al. 2000).

Before making other comparisons between our results and previous work, however, it should be noted that perhaps the most prominent disparity in EOG response patterns comparing rat and mouse involves isoamyl acetate (amyl acetate). This compound was used as a standard odor in the rat studies because it causes large and uniform responses in all parts of the OE (Scott et al. 1996). In the present study, isoamyl acetate had increasing response profiles as recoding location advanced from the caudal to rostral ends of all four turbinates that exceeded a twofold range for turbinate II' and III (Figs. 1*C* and 2). Clearly, if this odorant had been used as a standard in our study, it would have given biased results for all the other odorants. Since amyl acetate has previously been used as a standard and only has a middling HLC (Table 1), its response profile is not particularly probative concerning the sorption theory. However, carvone's response profile has been shown repeatedly to follow the predictions of the sorption hypothesis in the rat since it decreases down the airstream on turbinate IV (Scott et al. 2000). In the mouse, however, the carvone profile, like the case for most of the odors used in *experiment 1*, was highest in the middle of the turbinate (Fig. 2*D*). Limonene, a relatively insoluble compound, which had been shown in previous studies in the rat (Scott et al. 2000) to have an increasing profile (positive slope) moving caudorostrally along turbinate IV, showed a rather uniform profile, which had a nonsignificant slope in the mouse (Fig. 2*D*).

As a further test of the sorption hypothesis, we replotted the results of *experiment 1* using only the data from turbinate II and IV, the most upstream and downstream turbinates, respectively (Fig. 4*C*). Plotted in this way our data show variations in the response profile along the entire length of the airstream within the recorded region of the OE (Fig. 4*A*). While limonene still showed a flat profile, carvone had a significant overall decrease in response at the more downstream recording locations that was mostly a function of the decline along turbinate II (Fig. 4*C*). However, a comparison of slopes across the airstream for all the odorants in *experiment 1* failed to provide support for the sorption hypothesis, with only carvone having a significant slope. The results for isovaleric acid are particularly noteworthy since this is the most water-soluble (lowest HLC) compound in the study, with nearly 80 times greater water solubility than carvone. Contrary to the predictions of the sorption hypothesis, the response profile for isovaleric acid was flat along the entire airstream (Fig. 4*D*) and actually increased down the airstream along turbinate II (Fig. 2*A*). Moreover, mean responses on turbinate IV (Fig. 2*D*) did not decrease down the airstream nor were they consistently lower than those on turbinate II (upstream).

Experiment 2 was a more refined test of the sorption hypotheses since it employed odorants that were as follows: *1*) identical or similar to those used in previous sorption studies, *2*) near the extremes of the HLC scale (but see results above for isovaleric acid), and *3*) tested at two concentrations. However, neither nonane, a highly volatile hydrophobic odorant, nor benzaldehyde, a highly water-soluble odorant, showed a trend in responses across the recording locations on turbinate II (Fig. 4, *B* and *D*). On turbinate IV, both odors showed nonlinear trends, which were reminiscent of the results seen for most of the odors used in *experiment 1* in that the largest responses tended to be in the middle of the turbinate (Fig. 5, *C* and *E*; cf. Troitskaia 1988). Comparing the grand mean of responses from turbinate II with mean responses from different recording locations on turbinate IV did not provide evidence of a clear overall difference. This is relevant because the entirety of turbinate II is substantially upstream from any location on turbinate IV (see Fig. 4*A*). Only when we replotted the data along the entire airstream did we find a significant negative slope in the response profile for both concentrations of benzaldehyde (Fig. 5*F*). However, this result hinges on the mean responses of the most downstream recording location (IV.8), which if eliminated from the data removes the significant negative slope. It is suspicious that the two most downstream recording locations on turbinate IV show the lowest responses for both nonane and benzaldehyde, suggesting some kind of edge effect possibly caused by a thinning of the OE at the turbinate's rostral margin.

Taken together, our data provide little support for the hypothesis that a chromatograph-like spatial separation of odorants by the nasal cavity has influenced, through natural selection, the localization of OR subtypes in the mouse OE. To our knowledge, there have not been any studies of the imposed pattern of odor distribution in the mouse nasal cavity and our direct odor application method did not allow such measurements. We posit that the mouse's nasal cavity may be too small to bring about effective odor separation since decreasing the length and surface area of the stationary phase in a given chromatographic system lowers its resolution (Skoog et al. 2007, but see Jiang et al. 2010). Adams' (1972) compilation of the available morphometric data on nasal epithelial surface area in mammals suggests a stronger correlation with body weight than with a species' olfactory capabilities. More to the point, detailed morphometric measurements in the rat and mouse show that the mouse nasal cavity is nearly twofold shorter and its total surface area (including squamous, respiratory, and olfactory epithelium) is nearly fivefold lesser than the rat (Gross et al. 1982). While the mouse nasal surface-to-volume ratio is nearly double that in the rat (9.2/1 vs. 5.2/1), presumably increasing chromatographic potential, its much higher metabolic (and thus respiratory) rate suggests lower dwell times for inhaled odors decreasing chromatographic potential (Gross et al. 1982). Thus the chromatographic potential of the mouse's upper respiratory system may be orders of magnitude lesser than that in the rat and other similarly sized mammals.

However, even in the rat, the number of exceptions to the sorption hypothesis appears to be growing (Scott and Sherrill 2012). Apart from these empirical inconsistencies the fact that receptor zones are laid out in such a way as to encompass the most upstream and most downstream parts of the nasal airstream seems at odds with the hypothesis at a conceptual level (cf. Figs. 4*A* and 3*B*). Concerning sorption's potential role in discrimination, since all the ORs of a given subtype are arrayed across the entire longitudinal extent of a zone and converge onto a single medial or lateral glomerulus in the bulb, any information carried about sorption would be lost in this comingling of bulbar afferents. However, not addressed in our study is the potential for the medial and lateral paths within the ethmoturbinates to support a sorption effect (Scott et al. 1996). At least in this instance there are separate medial and lateral olfactory glomeruli to keep sorption information separate (but see Jiang et al. 2010). Nevertheless, it is difficult to conceive of how sorption information would meaningfully add to odor discrimination given the already staggeringly large dimensionality of rodent olfactory stimulus space afforded by >1,000 ORs (Wilson and Mainen 2006).

Olfactory maps. The study of the topographical organization of neurons in the nervous system, metaphorically referred to as "maps," has been a major current in neurobiology (Knudsen et al. 1987; White and Fitzpatrick 2007). For a sensory representation to be considered a map, in this sense of the term, it has been suggested that stimulus responses must be spatially clumped and there must be a systematic relationship between neuronal tuning and spatial position (Knudsen et al. 1987; Wilson and Mainen 2006). Classical examples of neural maps include the retinotopic and stomatotopic maps of visual and somatosensory cortices, respectively. In each case, these central maps recapitulate the spatial relationships of the cognate receptor organs: retina in the case of vision and dermal receptors in the case of somatosensation. Precisely because vision and somatosensation (other examples could be given) are fundamentally spatial sensory systems, it follows that nearest-neighbor relationships would be preserved at different levels of the afferent path to facilitate computations, such as contrast enhancement, by minimizing wiring length (Chklovskii and Koulakov 2004; Murthy 2011).

What could be the role, if any, of spatial dimensions in olfaction? Against the idea that spatial relationships matter in the olfactory system is the obvious lack of a spatial component in the stimulus. The mammalian olfactory system is sensitive to tens of thousands of different airborne chemicals that by the time they reach the OE are devoid of any spatial information (except concentration gradients). Nevertheless, the topic of spatial organization in olfaction has been vigorously pursed at least since the work of Adrian (1942, 1950). For example, afferent connections between the OE and olfactory bulb have been shown to be "rhinotopic" given that positions in the OE that sample the same internasal airspace tend to project to adjacent positions in the olfactory bulb (Schoenfeld and Cleland 2005, 2006). However, this organization only preserves course proximity relationships not a point-to-point mapping as in vision and somatosensation.

Perhaps the greatest preoccupation of those seeking a spatial organization of olfaction has been the search for "odotopic" and "chemotopic" maps in the olfactory bulb (Adrian 1942; reviewed by Johnson and Leon 2007; Murthy 2011). Odotopy, the proposition that individual odors activate distinct regions of the bulb, has been known to be valid, if trivially so, since the discovery that ORs have some level of ligand specificity and all ORs of a particular type project to a small number of glomeruli (Wilson and Mainen 2006). This kind of consistent pattern has been likened to a "look-up" table in computer software not

meeting the criteria of a true map as defined above (Murthy 2011). In contrast, the concept of chemotopy posits a systematic spatial representation in the olfactory system of some odorant feature such as carbon chain length or functional group (reviewed by Mori et al. 2006). While a great deal of evidence from a number of different laboratories (reviewed by Johnson and Leon 2007; Murthy 2011) using relatively low-resolution techniques support this canonical hypothesis, more recent high-resolution analyses suggest that even crude chemotopy does not exist in the olfactory bulb (Ma et al. 2012; reviewed by Murthy 2011). Indeed, a serious theoretical problem plagues the notion of spatial organization in the olfactory system: it is not clear how olfaction's tremendously high-dimensional stimulus space, entailing tens of thousands of odorants each with thousands of different physiochemical features (shape, size, polarity, etc.) could be mapped in the two dimensions available in the receptor sheet and glomerular layer of the olfactory bulb (Cleland and Sethupathy 2006). Based on the protracted but ultimately failed search for spatial organization in the bulb and more central regions of the olfactory system, there is no basis for the prediction that spatial organization exists in OE for the purpose of facilitating some central computation.

Another potential explanation for spatial patterns of response selectivity in the OE, at least at the gross anatomical level, emerges from recent studies of class I and class II ORs. These distinct groups have different distributions in the OE with the phylogenetically older class I ORs being almost exclusively limited to the dorsal recess (turbinate II in our study) and class II ORs being distributed throughout the OE (Zhang et al. 2004; Miyamichi et al. 2005). Even within the dorsal area of overlap the two classes of ORs maintain axonal segregation and innervate different parts of the olfactory bulb (Bozza et al. 2009). Based on patterns of bulbar response, it appears that the dorsal zone of the OE may be specialized for the detection of organic acids and other hydrophilic odorants consistent with class I receptor provenance from fish (Saito et al. 2009). Finally, genetic ablation of the dorsal zone OE abolishes innate fear responses but not learned fear responses to predator odors (Kobayakawa et al. 2007).

Our data provide only a modicum of support for the notion that the dorsal zone of the OE (turbinate II) is functionally distinct from the more ventral turbinates (II', III, and IV). For example, isovaleric acid, on average, caused a similar response magnitude in the dorsal and ventral turbinates (Fig. 2) and in *experiment 3* the putrid odorants (pyridine and ethane dithiol) were no more effective in eliciting responses in turbinate II than a floral (ethyl butyrate) or ethereal odorant (nonanol). However, isovaleric acid did show a significantly increasing rostrocaudal response profile in turbinate II that was flat or opposite in slope for the more ventral turbinates. None of the other odorant response profiles in *experiments 1* and *2* suggest that turbinate II was distinct compared with the ventral turbinates except that profiles in turbinate II tended to be more linear (Figs. 2 and 4).

Although limited by an exclusively medial approach, a modestly sized odor set, and the use of only two concentrations, our data are in agreement with previous studies showing spatially heterogeneous responses in the OE that are odor specific and consistent across animals (Mozell 1964; Mustaparta 1971; Mackay-Sim and Kesteven 1994; Scott et al. 1996, 1997, 2000; Scott and Brierley 1999). However, marked differences were found comparing our data to that in the rat, the only other mammal that has been studied in this detail (Scott et al. 1996, 1997; Scott and Brierley 1999; Scott et al. 2000, reviewed by Scott 2006). This may not be surprising given the hundreds of OR gene additions and deletions that have occurred since the phylogenetic split of rats and mice some 25 million years ago (Niimura and Nei 2007). Most of the variability we observed in EOG responses occurred in a direction perpendicular to predicted OR zonal boundaries, suggesting that regional OR specificity underlies our results. Specific tests failed to provide consistent support for the sorption hypothesis, and we found no evidence for sharp zonal boundaries or any suggestion of a true sensory map, although in the latter case its not clear how such a necessarily fractured map would be organized (Cleland and Sethupathy 2006). However, it seems doubtful that such a map would have the smooth contours observed in our data. Rather, we speculate that the spatial heterogeneity observed in mouse and other tetrapod OE responses is a function of a contingent evolutionary process that elaborated the complex and capacious olfactory system of land vertebrates from the more limited system found in fish. Indeed, the concentration of ORs with hydrophilic ligands in the dorsal zone may be an evolutionary artifact that could explain the OE mapping data in some species, which may only coincidently be consistent with the sorption hypothesis (Miyamichi et al. 2005).

Lord Adrian, later in his scientific career (1954), came to grips with the fact that space may not be as important in the olfactory system as he originally thought when he stated that ". . . neighboring groups of olfactory receptors have marked differences in their sensitivity to different smells. I have no doubt, therefore, that olfactory discrimination is not due solely to . . . the deposition of material in different patterns on its surface owing to differences in air flow, size of molecule, etc." Despite the decades of effort since Adrian's original work trying to prove otherwise, we now have ample reason to conclude that olfaction is fundamentally a nonspatial sensory system.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: D.M.C. and S.M.R. conception and design of research; D.M.C., C.T.W., S.M.R., and D.A.B. performed experiments; D.M.C., C.T.W., and S.M.R. analyzed data; D.M.C., C.T.W., S.M.R., and D.A.B. interpreted results of experiments; D.M.C., S.M.R., and D.A.B. prepared figures; D.M.C. drafted manuscript; D.M.C. and C.T.W. edited and revised manuscript; D.M.C., C.T.W., S.M.R., and D.A.B. approved final version of manuscript.

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