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Research report

Mucosal activity patterns as a basis for olfactory discrimination: comparing behavior and optical recordings

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Abstract

In over half a century numerous studies have demonstrated that different odorants produce individually different spatial patterns of neural receptor activity on the olfactory mucosa. However, the thought that these differential activity patterns could be the neural code underlying olfactory perception has not been tested directly. In the present study using operant techniques, rats were trained to differentially identify five odors from a homologous series of iso-intensive straight-chain aldehydes that differed serially by only one carbon atom, viz. hexaldehyde to decaldehyde. The rats identified each of the five odorants with greater than 90% correct identification. The degree of perceptual similarity between any pair of the five odorants was determined. Using multidimensional scaling techniques (MDS) the similarity measures yielded a two-dimensional perceptual odorant space. Optical techniques were used to record the olfactory mucosal activity patterns in response to these same five iso-intensive aldehydes. The mucosal activity elicited by each odorant revealed individually distinct band-like patterns that varied both within and across these bands. More importantly, the relative differential responsivity of the bands was related to chain length. An MDS analysis of the dissimilarity measure between all possible pairs of odorant induced activity patterns yielded a two-dimensional neurophysiologic odorant space. Further analysis indicated that the neurophysiologic and psychophysically determined odorant spaces were highly correlated ($F_{1,39}=23.9$, $P=$nil). These results give additional credence to the concept that the odorant-induced mucosal activity patterns may serve as the substrate for the perception of odorant quality.

Theme: Sensory systems

Topic: Olfactory senses

Keywords: Optical imaging; Odorant quality perception; Behavior; Mucosal activity pattern; Rat

1. Introduction

Thanks to a series of ground breaking studies using cell and molecular techniques [3,4,22,28] the current, most generally accepted view of how different odorants are neurally represented at the olfactory mucosa for further analysis centrally is, in summary, as follows: each olfactory receptor cell is endowed with a small number, most likely one, of different odorant receptor (OR) types each of which is tuned to a different broad range of chemicals, which is presumed to reflect the molecular response range of the OR itself. There appears to be as many as a thousand differently tuned receptor types, and, most importantly, all the neurons bearing the same receptor type uniquely send their axons to the same glomeruli in the olfactory bulb. Thus, at the level of the mucosa each odorant would be represented by a unique set of excited receptor neurons that, in turn, at the level of the bulb would be represented by a unique combination of activated glomeruli. Note that in this current, generally accepted view no relevance for representing odorants is given to the spatial positions occupied by the activated sets of neurons on the mucosa.

This neglect of the mucosal activity in spatial terms is in sharp contrast to what was probably the previous most generally accepted view of how different odorants are neurally represented at the level of the mucosa. This earlier view actually highlighted the patterns of spatial activity

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across the mucosa, in this regard. This view was based upon the reports of differential odorant-dependent spatial activity patterns across the mucosa by a long list of investigators who, taking their lead from Adrian [1,2], used a variety of electrophysiologic techniques and a number of different species in confirming the existence of these differential patterns [6,7,9,10,13,15–17,19,20,25,27,32]. In addition, some of these studies also reported different odorant-dependent temporal patterns, thus fulfilling Adrian's seminal proposition that odorants are represented at the mucosa by the different spatiotemporal activity patterns they produce. This view of odorant representation at the mucosa gained support, albeit somewhat tacitly from some investigators, not only because the patterns were so repeatedly demonstrated but also because no study had been able to demonstrate finely tuned, ligand-specific receptor neurons which, it was generally agreed, would be a much more parsimonious way to represent the odorants. With the more recent demonstrations of molecular response range specific receptor neurons which project to specific glomeruli in the bulb, the collective expert opinion has come to favor this much more parsimonious process for the representation of different odorants over the highly complicated spatiotemporal activity patterns.

However, although this view has become favored over the odorant spatiotemporal activity patterns for the representation of different odorants at the level of the mucosa, it should be emphasized that the existence of the odorant-selective mucosal activity patterns has not been questioned. They do exist, and so far the worst that can be said of them, as far as representing odorants are concerned, is that they might be artifacts, i.e., they might be a necessary consequence of the way some receptor types are distributed on the mucosa but play no direct part in the chain of events leading to the perception of different odorants. On the other hand, these spatiotemporal patterns might actually play some as yet unidentified role in the perception of odorants, perhaps, for instance, by modulating the patterns of glomerular activity developed in the bulb by the specifically activated receptor neurons. Yet again, the mucosal spatial differentiations could be an early process in the more complete spatial analysis now known to exist at the level of the bulb.

Thus, before concluding that these well-documented mucosal activity patterns are 'artifacts', their relationship, or lack thereof, to the perception of odorants should be investigated. In developing such an investigation, it is reasonable to consider what parallels might be expected between the mucosal patterns and behavioral perception if the two are actually related. One might expect, for instance, that the more alike two odorants appear in behavioral judgments the more alike would be their activity patterns recorded from the mucosa. Another reasonable expectation is that the resolving power of differentiating odorants behaviorally should be matched by the differential resolution between their mucosal activity patterns. Furthermore, one might expect that the neurophysiologic patterns developed on the mucosa, to some degree, should follow the spatial receptor distribution described by numerous authors, using cell and molecular techniques. With this purview in mind, the present study represents an early attempt to uncover the role, if any, of odorant induced mucosal spatiotemporal activity patterns to the perception of odorants.

Although many studies have demonstrated the presence of these different odorant-selective mucosal spatiotemporal activity patterns, only two studies have questioned whether their mere presence is enough to conclude that they actually play a role in the perception of different odor qualities.

Bennet [5] tested whether rats could still recognize previously learned odors when the mucosal activity pattern each odorant evoked was presumably altered by redirecting its mucosal flow with nasal baffles. Unfortunately, this study lacked the critically needed evidence that the baffles did, indeed, alter the activity patterns. Therefore, the Bennet study is better cited for the first to raise and address the question rather than for the data collected.

The authors of the second study [11] reasoned that to consider these activity patterns as basic to the perception of odorant quality, there should be some systematic relationship between the perceptual quality of the odorants and the activity they elicit on the mucosa. Using optical recording techniques to map the mucosal locations of each odorant's peak activity and, for the same odorants, using the number of confusion errors in a behavioral identification task to assess odorant similarities, the authors observed a striking parallel between the quality differences among the odorants and the relative positions on the mucosa of the activity they produced. Thus, this study met the authors' condition for considering the differential activity patterns as basic to the perception of different odorants. On the other hand, the odorants used in the study differed very widely from each other both chemically and perceptually. It could be argued therefore, that it did not test the adequacy of the mucosal activity patterns as a neural basis for the perception of odorant quality at the level of fine resolution for which the olfactory system is well noted. A more convincing examination of the activity patterns as a neural basis for olfactory perception would be to use a series of similar odorants thus putting to the test whether the resolution among the activity patterns they produce matches that of the olfactory system.

To meet this challenge, the present study used five odorants from the same homologous series with each one differing from its predecessor in the series by one carbon atom which, unlike in the previous study, varied the odorants with a precise and easily recognized minimal perturbation. If with this series of relatively minimal changes in molecular structure some aspect of the behavioral perception of the odorants varied systematically with some aspect of the activity patterns they produced,

this would give strong indication that the activity patterns may yet be basic to the differential perception of odorants.

2. Materials and methods

2.1. Psychophysics

The training procedures used to shape the odorant identification task were identical to those previously reported by Youngentob and co-workers [30,31]. Using standard operant techniques 10 adult Long–Evans Hooded rats were trained to differentially report (i.e., identify) odorants in a Plexiglas arena containing an odorant sampling port and five response tunnels. Each tunnel 'represented' the response location for one of the homologous series of aldehydes (hexaldehyde, heptaldehyde, octaldehyde, nonaldehyde, and decaldehyde). Odorant and response tunnel associations were randomly assigned for each rat. The concentrations of all the odorants were chosen such that they were judged by laboratory personnel to be equally intense and well above threshold. (Note, in this regard, when odorant concentration is well above threshold, odorant intensity is not a salient feature of the identification process [30]). The concentrations, in terms of percent of vapor saturation at 20 °C, were 0.38, 0.88, 1.65, 7.5, and 2.25 for hexaldehde, heptaldehyde, octaldehyde, nonaldehyde and decaldehyde, respectively. In short, the rats were trained to enter the sampling port and sniff the odorant presented and then leave the stimulus sampling port, in order to choose the response tunnel 'representing' the odorant. A response choice was reported when the rat licked a reinforcement cup at the end of one of five response tunnels. Odorant stimuli were randomly presented in blocks of five trials and testing continued for 40 consecutive blocks. Each rat contributed 18 testing sessions to the data.

2.2. Neurophysiology

Using previously established methods [12], the turbinate and septal olfactory mucosal activity patterns from another set of 10 Long–Evans Hooded rats were recorded, using optical techniques and the voltage sensitive dye di-4-ANEPPS. After the mucosa was surgically excised it was placed in a Delrin chamber and covered with a clear plastic plate. The distance between the mucosal surface and the plate was 3–4 mm thereby, minimizing any possible chromatographic effect upon the distribution of odorant molecules [21]. The Delrin chamber had an input and output port on opposite sides of the chamber. The input port was connected to a T type connector. Odorized or deodorized air continuously flowed at 600 ml/min through the connector bypassing the chamber. By continuously applying a negative pressure to the output port, a constant flow of air at 250 ml/min was drawn across the mucosa. During stimulation, odorized rather than deodorized air flowed through the T connector for one second. The methods were identical to those previously described by Kent et al. [12] except that the fluorescence changes were monitored with a Dalsa (CA-D1) 12 bit, 120×120 digital area scan camera at 40 frames/s. Moreover, while we maintained our prior temporal filter of a 0.5 s running average, for the present study we added a Gaussian spatial filter (SD=3 pixels) to the pattern analysis.

The mucosal activity patterns in response to the same five odorants used in the behavioral tests were recorded from each rat's septal and medial turbinate surfaces. The approximate recording area was 6 mm×6 mm. The concentrations for all the odorants were chosen such that the size of the elicited responses were about equal and were within the concentration–response dynamic range. The percentages of vapor phase concentration were 4.5, 7.0, 15.0, 36.0 and 60.0% for hexaldehde, heptaldehyde, octaldehyde, nonaldehyde and decaldehyde, respectively (Note: the stimuli indicated were the nominal concentrations under the artificial conditions of our stimulating procedure required to achieving our response parameters. Based on the internal volume of the stimulating chamber, the distance between the epithelium and the clear plastic plate, the diameter of the input nozzle, and the stimulus flow-rate, we estimate that the effective odorant deposition at the olfactory epithelium is equivalent to that achieved by presenting a 10- to 100-times smaller concentration of odorant to an intact behaving animal; Scherer, 2003 [24]). For both mucosal surfaces each odorant was presented twice in a randomized Latin Square block design to minimize any bias that might occur from the order of odorant presentation. A standard stimulus of amyl acetate at 1.0% of vapor saturation was given at the beginning, end and between blocks to adjust for changes over time in mucosal sensitivity.

3. Results

3.1. Neurophysiology

Fig. 1 shows the targeted areas of the turbinate and septal mucosas sampled in each rat by the optical recording system. Note that the orientation of these areas on the opposing turbinate and septal mucosas are the same and in register relative to the nasal flow path with, however, the area on the septum slightly closer to the cribriform plate. This figure defines the mucosal areas sampled in all the subsequent figures of mucosal activity.

As examples of the topographical 120×120 pixel array of recorded responses, Fig. 2 shows one array (blue-scale) in response to the standard, amyl acetate and another in response to hexaldehyde, on the turbinate mucosa. In this figure the optically recorded response trace for every fourth pixel of the digital area scan camera are superim-

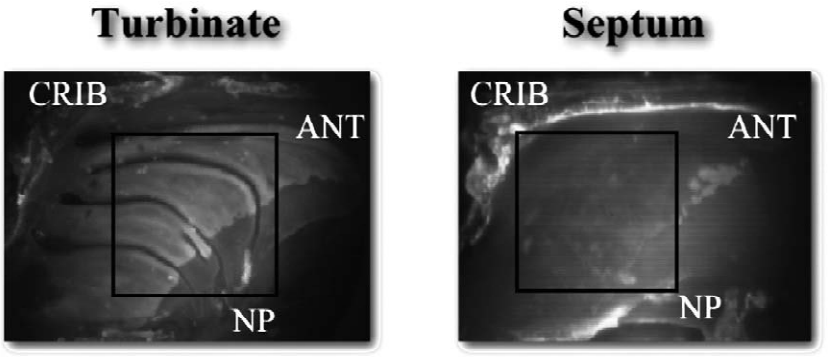

Fig. 1. Mucosal recording sites. The boxes indicate the approximate recording area. The two opposing halves of the nasal cavity were brought into register by rotating the septal image around its horizontal axis. The small patch of weak fluorescence in the lower right corner of the box is respiratory mucosa. CRIB, ANT, NP refer to cribriform, anterior and nasopharynx, respectively.

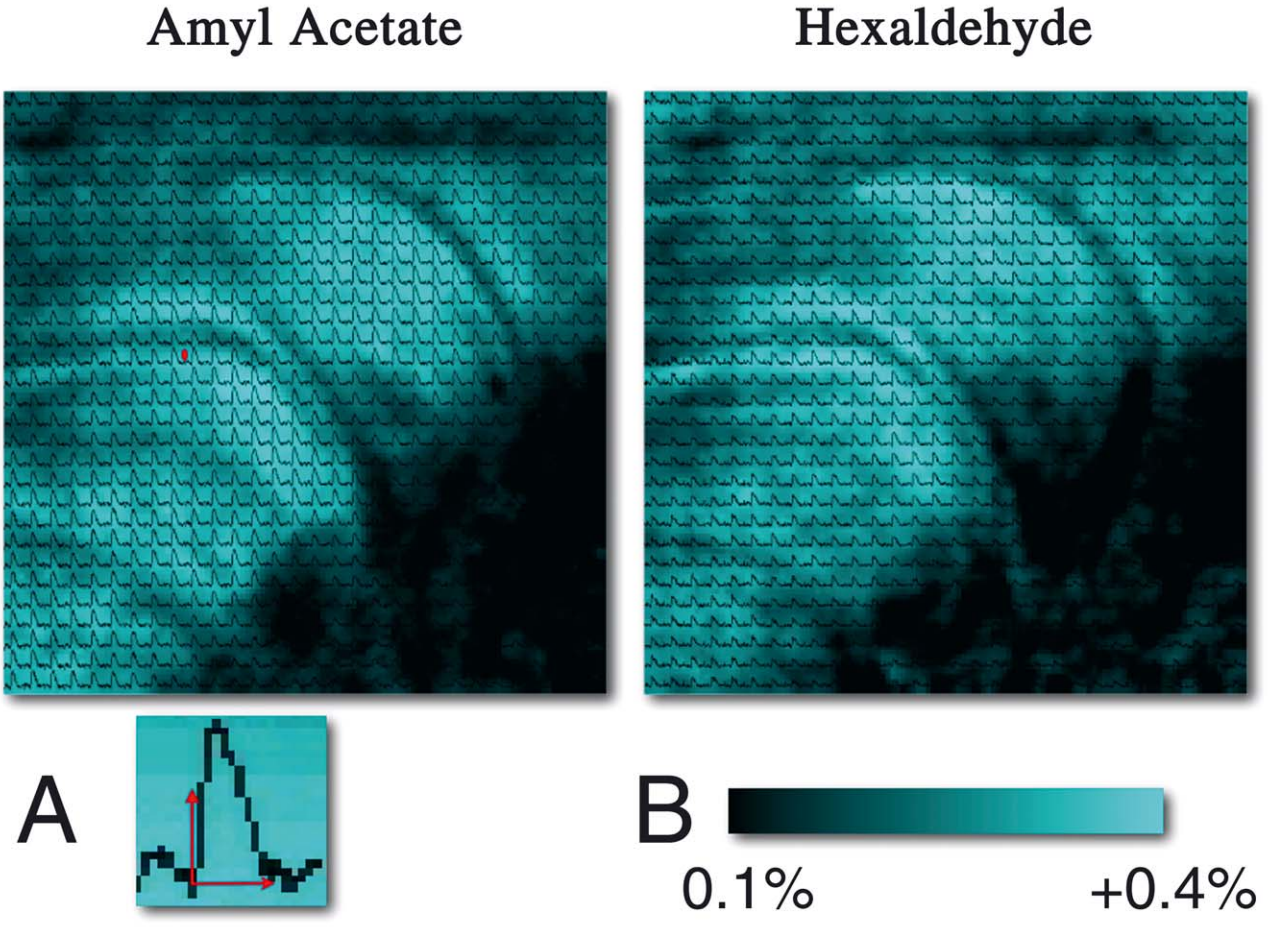

Fig. 2. Optical signals recorded from the turbinate mucosa. The optically recorded response traces are shown for every fourth pixel of the digital area scan camera. The length of each tracing is 45 s and the peak to peak height is 0.5% change in fluorescence. These are superimposed upon a blue-scale representation of response magnitude for all the pixels. The larger the response the brighter the pixel. Although both odorants give responses across the entire sampled area of the mucosa, there are two broad regions of differential response; upper left hand corner versus a diagonal band-like area. Panel A illustrates a magnified view of the pixel indicated by the red dot in the amyl acetate array. The vertical height of the red arrow in the panel represents 0.25% change in fluorescence and the horizontal arrow indicates 20 s. The beginning of the time arrow is the onset of the stimulus. Panel B illustrates the continuum of blue-scale response magnitude with black set to 0.1% change in fluorescence and bright blue to 0.4%. (Note: the spatial filter applied to the responses for this figure only included the pixel of interest plus the immediately neighboring pixels).

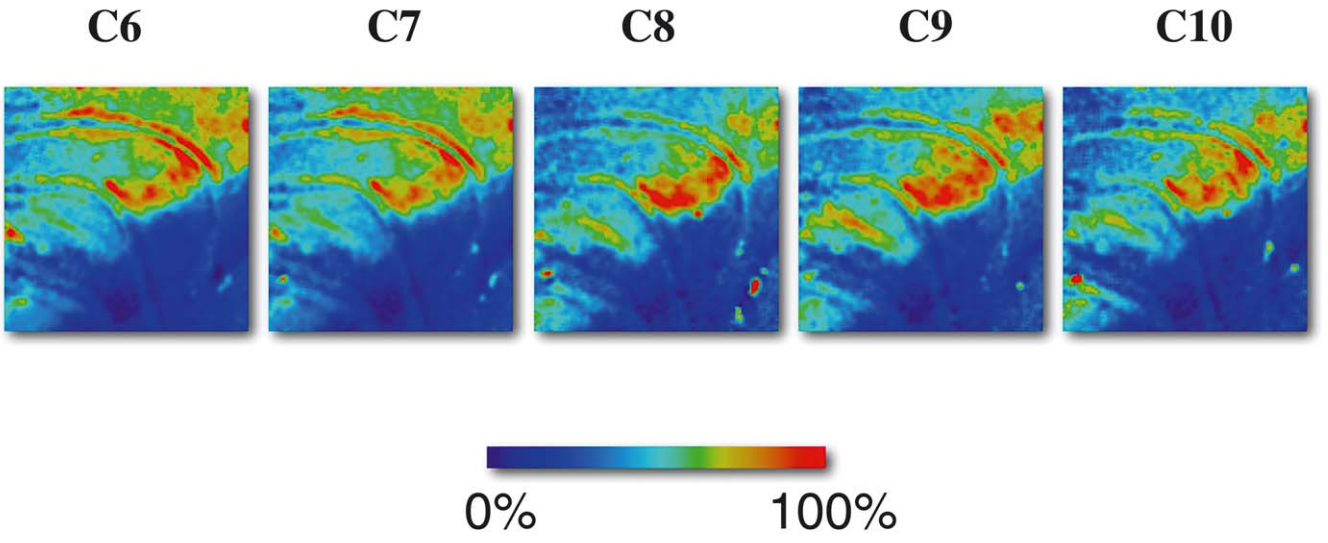

Fig. 3. Normalized individual turbinate response patterns for hexaldehde (C6), heptaldehyde (C7), octaldehyde (C8), nonaldehyde (C9) and decaldehyde (C10), respectively. As illustrated by the color scale, for any given pixel of the 120×120 array, blue indicates no response whereas red denotes a relative response equal to 100%. The five responses are from the same animal illustrated in Fig. 2. Although the patterns for each odorant were similar (as might be expected with a homologous series), a change in responsivity across the epithelium can be appreciated in its fine detail. For example, focusing on the center of each panel (corresponding to the tip endoturbinal II), there is an increasing relative response for C8 and C9, followed by a smaller response for C10. (Note: the spatial filter applied to the responses for this figure only included the pixel of interest plus the immediately neighboring pixels).

posed. By contrast, Fig. 3 represents the pseudo color topographical response profiles for the 120×120 array in response to each of the five odorants for the same animal illustrated in Fig. 2. Note that each of the odorants elicited responses from the entire area of the sampled mucosa, however, as quantified and detailed below, in different band-like patterns of relative activity. To minimize any possibility that differences in mucosal sensitivity might subvert the differences in the activity patterns, the overall odorant responsivity of each mucosa was normalized to unity [10].

To highlight these bands of activity in any single animal to any single odorant, the average response size in a given pixel for the odorant in question was divided by that same pixel's average response size across all five odorants. This procedure, which expresses the difference in response magnitude as a ratio, is repeated for all 14400 pixels of the camera array. Each ratio value for a given pixel was color coded such that pink represented a pixel in which the average response for the odorant of interest was larger than the average response across all five odorants (ratio >1). Further, the more saturated the hue the greater was the average response for the odorant of interest over that across all five odorants. In contrast, blue depicted a pixel in which the average response for the odorant of interest was less than that across the five odorants (ratio<1) and the more saturated the blue the greater was the average response across all five odorants relative to that of the odorant of interest.

When all 14400 of these color-coded pixels were laid out topographically, representations of the differential mucosal activity, like those shown in Fig. 4, and in several later figures, were generated. Each of the five mucosal activity patterns shown in this figure were recorded from the turbinate mucosa of a different rat in response to the same odorant, hexaldehyde.

This figure fulfills several purposes. First, it introduces the band-like spatial patterns developed across the mucosa by the odorants. Second, it shows reasonable similarity in the differential response patterns elicited by the same odorant in five different animals. Finally, it illustrates non-uniformities within the bands. That is, for the area of relatively larger responsivity for hexaldehyde (pink) there are regions which are much less saturated. Likewise, for the area of relatively lesser responsivity for hexaldehyde (blue) there are regions of less saturated blue. It is clear from Fig. 4 that although the general differential pattern of activity elicited by a given odorant in the five different animals was certainly similar, there was some variation among them in the fine detail.

In addition to differential mucosal band-like activity patterns based upon response magnitude, there were also similar band-like activity patterns based upon response latency such that the larger the response magnitude the shorter the response latency, as shown by comparing Fig. 4 to Fig. 5.

Fig. 6 shows how the carbon chain length in a homologous series of aldehydes (C6 to C10) affects the average mucosal activity patterns recorded from both the septal and turbinate mucosas. In both rows two activity bands (an upper or more dorsally located band and a lower or more ventrally located band) are seen in each display. In the first display of the septal response (i.e., C6) the upper band is pink and the lower band is blue, meaning that the response

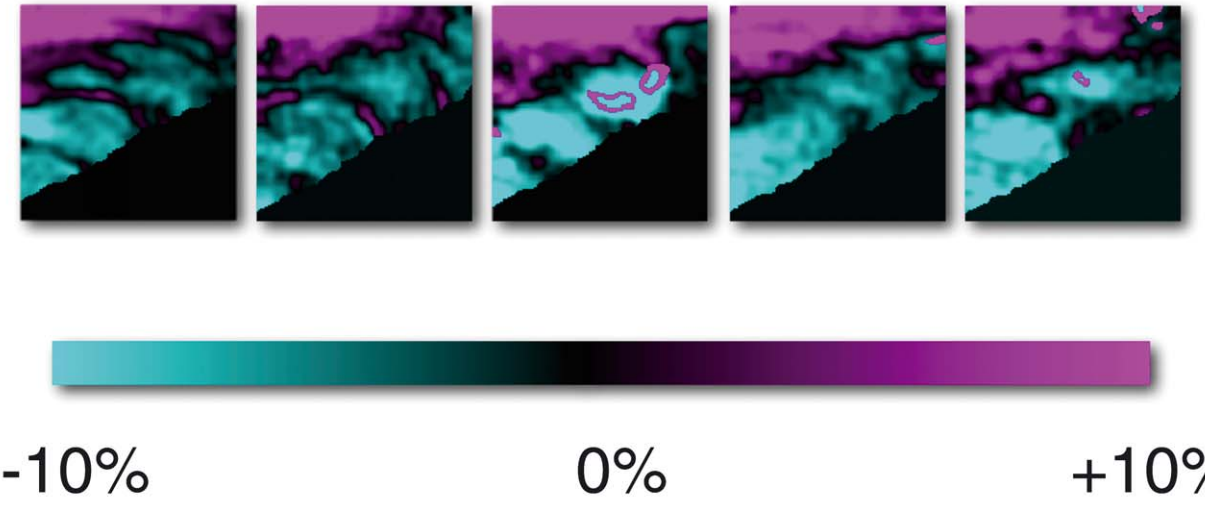

Fig. 4. Individual turbinate response patterns for hexaldehyde (C6) from a random sample of five experimental rats. The orientation of the turbinate is the same as in Fig. 1. Saturated pink indicates that the response to C6 is 10% larger than the response averaged across all five odorants and saturated blue is 10% less. The color bar represents the continuum of response between the saturated endpoints with black set to zero.

to the odorant of interest (C6) was greater than was the average response across all five odorants on the upper band but less compared to the average response across all five odorants on the lower band. In the next display (i.e., C7) the bands shift colors, indicating that there was a marked shift in the responsivity of these bands going from C6 to C7 with the lower band now becoming more responsive to the odorant of interest. However, for C8 and C9 the pink and blue become increasingly less saturated and more interdigitated, indicating that there was an increasing trend toward an equal responsivity of both bands to the odorant of interest compared to the response across all five odorants. In the last display (C10) there is a return to the relatively greater responsivity of the upper band to the odorant of interest. A reasonably parallel relationship holds in Fig. 6 for the turbinate mucosa, but in this case the increment in the relative response to the odorant of interest from nonaldehyde to decaldehyde, though still present, was less pronounced. Thus, for the upper band there appears to be a progressive shift in relative response magnitude from greater to lesser and back to greater as chain length is increased. By contrast, the reciprocal is true in relative response magnitude for the lower band. Thus, by visual inspection it appears that odorants differing from each other by but one carbon atom in a homologous series can produce differential changes in the activity elicited across the olfactory mucosa.

To quantify these differences, the following measure of pattern similarity was devised. First, each odorant's pattern was quantitatively described by averaging the responses in corresponding pixels across the two presentations of odorant. Next, a correlation coefficient between corresponding pixels of any two patterns was determined. With five odorant patterns for each of 10 animals, there was a 50×50 matrix of correlation coefficients indicating pattern similarities. These were subjected to an MDS analysis that yielded a two-dimensional odorant space upon which the odorants were arranged according to their relative spatial response similarities.

Fig. 7 plots the relative position of each odorant in the odorant space. As can be seen in this figure, as aldehyde carbon length increases the odorant positions trace out a sequential U-shaped relationship. The 50 x and y coordinates were then subjected to a randomized block multivariate analysis of variance (MANOVA). The x, y coordinates were the dependent variables and odorant was the independent variable. The multivariate null hypothesis that these patterns were not different was rejected with the Wilkes/lambda likelihood test of $F_{8,8}=103$ and $P=$nil. Note that the spatial coordinates for each odorant were quite consistent and significantly different from one another as documented by the MANOVA. The result supports, at least based upon this set of aldehydes, the notion that very structurally similar odorants can produce differential patterns of mucosal activity.

3.2. Psychophysics

Fig. 8 shows the composite odorant confusion matrix for 10 rats. The far left column gives the odorants presented and the uppermost row represents the odorant identified in response to the odorant presented. Thus, C6 was identified as C6 92.1% of the time, as C7 4.3%, as C8 1.2%, as C9 1.2%, and as C10 1.2%. Similar entries were made for the other aldehydes. Clearly, the animals were capable of differentially reporting the five odorants with a high degree of accuracy (greater than 90%), even though the aldehyde odorants differed sequentially by but one carbon atom. However, as can also be seen, there were misidentifications

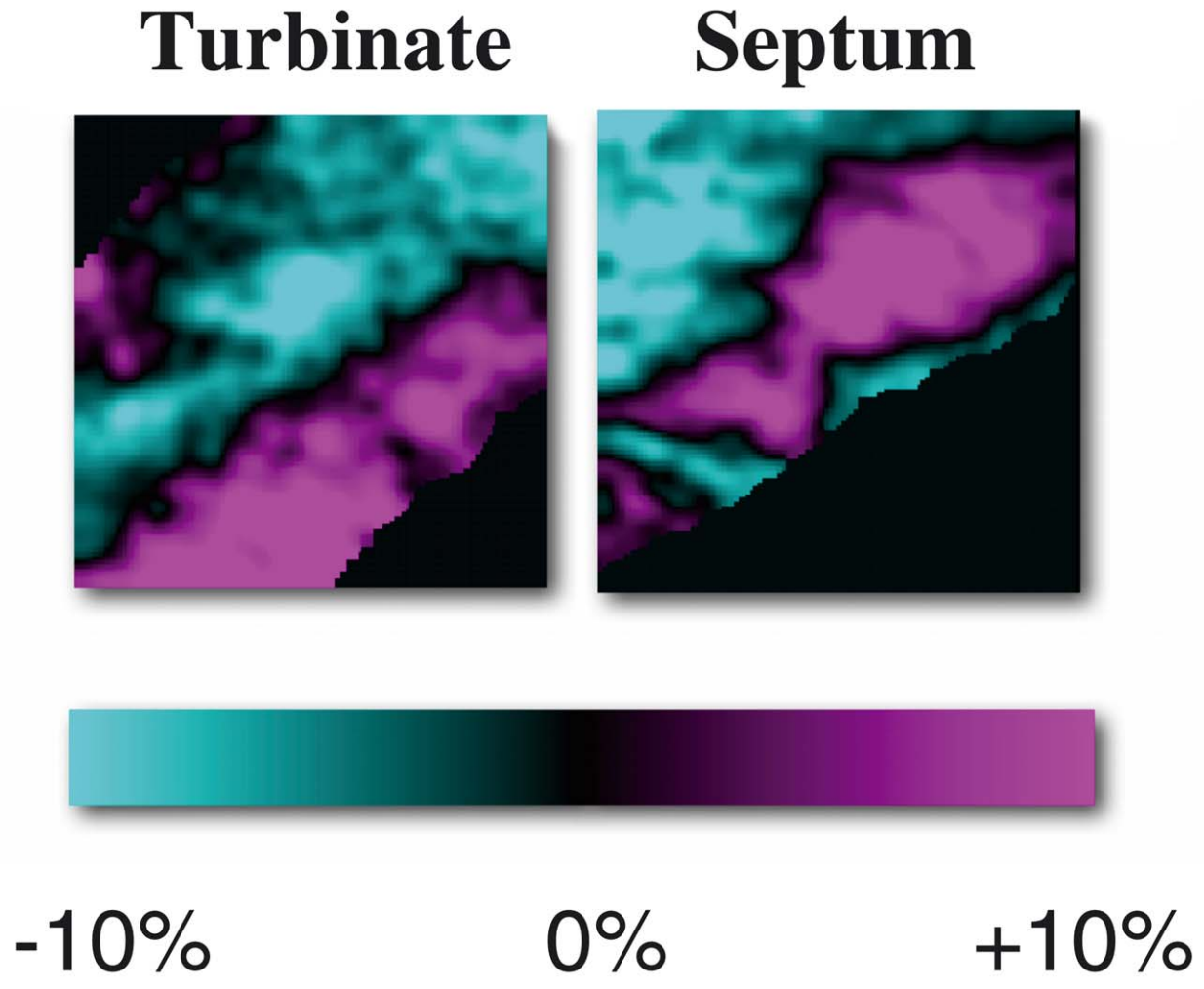

Fig. 5. Composite start time patterns for hexaldehyde (C6) averaged across all 10 rats. The more saturated the blue area the shorter is the response latency to C6 and the more saturated the pink area the longer the latency to C6. The orientation of the turbinate and septum is the same as in Fig. 1. Saturated pink indicates that the response to hexaldehyde (C6) is 10% longer than the response averaged across all five odorants and saturated blue is 10% shorter. The color bar represents the continuum of response between the saturated endpoints with black set to zero. (Note: the start of the response was defined to occur when the leading leg of the response was equal to $1/e$ of the peak).

such as C6 being identified as C7 4.3% of the time but only confused as C8 1.2% of the time. This was taken to mean that, to the rats, C6 was perceptually more similar to C7 than to C8.

In order to determine whether these behaviorally derived similarities paralleled the similarities of the neurophysiologic patterns, an odorant space based upon these behavioral data was constructed. The following procedure was used: first, in order to equalize the weightings of each of the rows the summed misidentifications for a given odorant (row of responses) were normalized to a value of 100. Next, each pair of reciprocal similarities (e.g., misidentification of C7 for C6 and C6 for C7) were then averaged and the resulting matrix of similarities was subjected to an MDS analysis. An odorant space was calculated for each of the 10 rats yielding a two-dimensional space upon which the odorants were arranged according to their relative spatial response similarities.

In order to standardize the arbitrary orientation of the MDS space, each animal's individual odorant space was flipped and rotated to optimize its alignment to the averaged neurophysiology odorant space discussed above [11]. Fig. 9 plots the averaged relative position of each odorant in this behaviorally determined perceptual space along with its standard error (ellipses around the data points). This plot demonstrates no overlap among the odorants, showing, as might be expected from the high level of correct identification, that the odorants were perceptually different. Of particular interest is the finding that the perceptual odorant space (Fig. 9) shows a marked similarity to the prior neurophysiologic odorant space (Fig. 7) in that both odorant spaces trace out a similar sequential U-shaped relationship with increasing aldehyde length. Furthermore, on their respective x-axes both odorant spaces show a relative maximum at C8 and minima at C6 and C10.

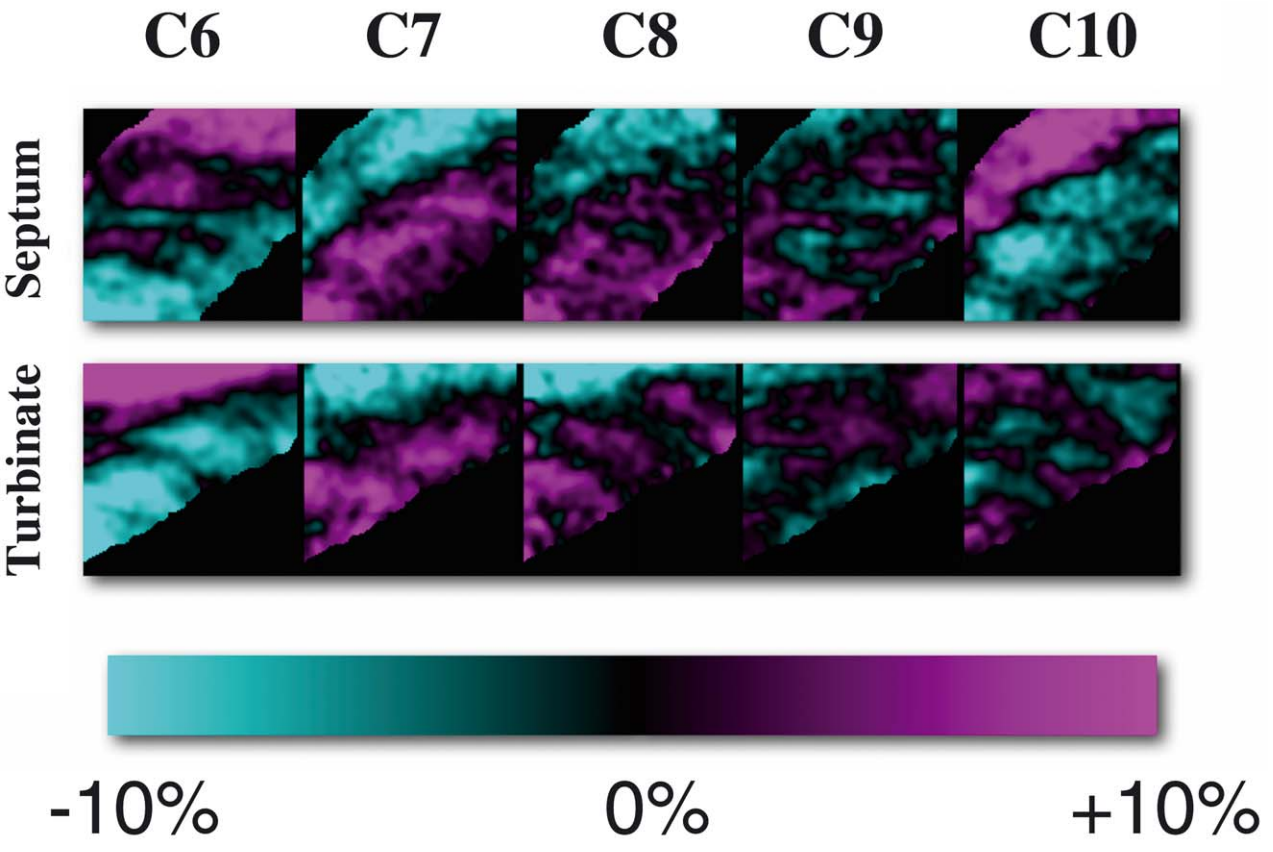

Fig. 6. Composite response patterns averaged across all 10 rats. The orientation of the septum and the turbinate is the same as in Fig. 1. Saturated pink indicates that the response for the odorant of interest is 10% larger than the response averaged across all five odorants and saturated blue is 10% less. The color bar represents the continuum of response between the saturated endpoints with black set to zero. C6, C7, C8, C9, and C10 denote hexaldehde, heptaldehyde, octaldehyde, nonaldehyde and decaldehyde, respectively.

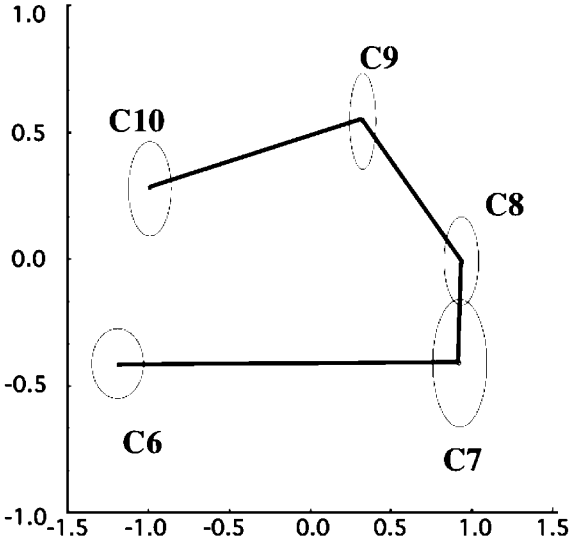

Fig. 7. Neurophysiologic odorant space for all five aldehydes determined from all 10 rats, using multidimensional scaling techniques. The average and standard error in each dimension are shown. See text for details.

Stimulus Response Alternatives

Stimulus	C6	C7	C8	C9	C10
C6	92.1	4.3	1.2	1.2	1.2
C7	3.1	92.2	2.2	1.0	1.5
C8	1.4	3.6	93.3	1.3	0.4
C9	1.5	0.9	0.2	97.1	0.3
C10	1.4	1.5	0.5	0.8	95.8

Fig. 8. Composite rat odorant confusion matrix. The stimuli presented are listed along the left margin. The response alternatives, which could possibly be chosen for each stimulus, are listed along the upper margin. Each entry gives the average number of times (in percentages) that each of the alternatives was chosen for each of the stimuli. Each odorant was presented a total of 720 times to each rat.

3.3. Comparison of neurophysiologic and psychophysical results

In qualitatively comparing the perceptual odorant space

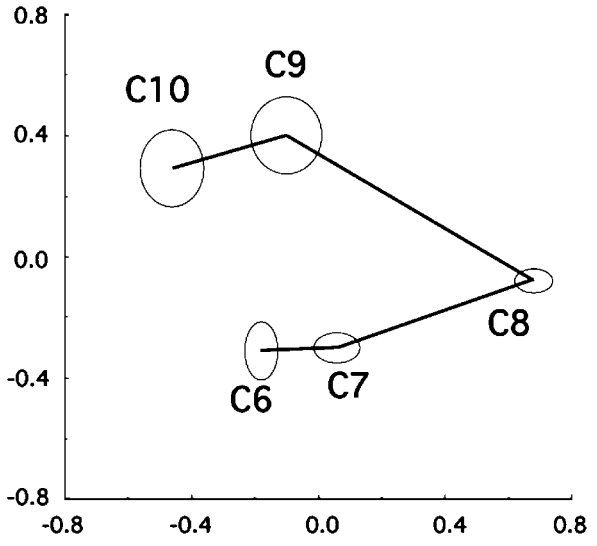

Fig. 9. Perceptual odorant space determined from the composite behavioral data from 10 rat odorant confusion matrices, using multidimensional scaling techniques. The average and standard error in each dimension are shown. See text for details.

to the neurophysiologic odorant space there appeared to be a marked correspondence as well as obvious discrepancies. For example, the graphical distance between C6 and C7 was much closer behaviorally than electrophysiologically. Therefore, to test the overall correspondence between the behavioral and electrophysiologic spaces a two-dimensional pattern regression analysis [32] was performed. This analysis calculated an F ratio based upon the correlation coefficients between the coordinate points of each rat's perceptual and neurophysiologic odorant space. The calculated R^2 was 0.38 and the F ratio with 1 degree of freedom in the numerator and 39 in the denominator was 23.9 ($P = \text{nil}$). This analysis demonstrated an appreciable relationship between the behavioral and neurophysiological odorant spaces.

4. Discussion

An interesting ancillary observation in this study was the band-like configurations of the mucosal activity patterns in response to the different odorants. These band-like configurations are reminiscent of the zonal distributions of different receptor types reported by a number of investigators, cited previously, using cellular and molecular techniques. However, speculation that the bands recorded neurophysiologically in the present study actually reflect the zonal distribution of receptors reported earlier, must await a study specifically designed to address that hypothesis. Nevertheless, the present study does raise questions about the anatomical distribution of receptors which, if the bands in this neurophysiologic study do represent the zones in the previous molecular studies, should be taken into consideration. Specifically, although the present neurophysiologic data may appear consistent with the concept that receptors are arranged in zones, they are not consistent with the concept that like receptor types are necessarily distributed homogeneously within a zone. Certainly, the response patterns in Fig. 3 and the color saturation of the differential response bands in Figs. 4, 5, and 6 are far from homogeneous. In this regard, it should be noted several studies suggest that receptor types are not necessarily distributed homogeneously within a zone. For instance, members of the OR37 odorant receptor family have been shown to have a clustered distribution pattern [26]. Moreover, recent in situ hybridization data suggests that odorant receptors previously held to be randomly distributed within a given zone are instead aggregated into a particular region [8], although in a much less punctate manner than the OR37 receptors. Therefore, the neurophysiologic activity patterns produced by different odorants at the mucosal level in this study may not only reflect the zonal organization of receptor types but also their differential aggregation within a zone.

More pertinent to the main purpose of this study were the complementary findings that even with a minimal carbon length change of one atom in a homologous series of aldehydes, rats were able to discriminate among them and each odorant in sequence produced a differential pattern of spatiotemporal mucosal activity. Of particular importance was the finding that the mucosal activity patterns and the perception of these aldehydes were correlated. That is, the greater the similarity between the neurophysiologic patterns produced by any two aldehydes, the more they were perceptually confused. Consequently, for these aldehydes, there appears to be a significant relationship between their mucosal activity patterns and their behavioral perception.

The fact that the behavioral data so closely paralleled the neurophysiological data is particularly notable considering the number of contingencies that could have masked this parallel between the two measures altogether and, short of that, are probably the bases for whatever discrepancies do exist. One such contingency was the fact that the neurophysiological recordings were, of necessity, taken from a limited area of the mucosa whereas the behavior was presumably based upon information coming from the entire mucosa.

Yet another contingency was the fact that the patterns on the mucosa occur before, and cannot benefit from, the sharpening which occurs as the receptor cell axons project to the bulb. A number of studies [18,23,29] have shown that axons of like receptor types project to a limited number of bulbar glomeruli, a process which could further refine the mucosal spatial patterns for subsequent behavioral discrimination. Furthermore, as shown in the present study, those bands which were relatively more responsive to an odorant also responded more rapidly, and this temporal difference could further sharpen the patterns at the bulb by facilitating lateral inhibition. Indeed, Linster

et al. [14] demonstrated a similar but perhaps even more refined relationship between evoked neural activity in the bulb and perception. That is, the degree of similarity between the neural representation of enantiomer pairs predicted their perceptual similarity. Nonetheless, in the present study even though the bulbar sharpening which was presumably available for the behavioral discrimination of odorants had not yet occurred at the mucosal level, a robust relationship between the mucosal patterns and the behavioral discriminations was still quite apparent. This relationship is consistent with the concept that the mucosal activity patterns could possibly serve as a neural substrate for the behavioral discrimination of odorants.

The present study confirms the observation of Kent et al. [11] that there was a parallel between the ability of rats to discriminate behaviorally among odorants and the differences in the mucosal activity patterns produced by those odorants. However, in the present study this relationship was tested using much more stringent conditions. Specifically, the odorants used differed by but one carbon atom in a homologous series, thereby requiring a level of resolution, both for the behavioral discrimination and for the mucosal activity patterns, that would be expected if the latter did, indeed, underlie the former. In addition, by varying the odorants in the orderly fashion afforded by a homologous series differing serially by a single carbon atom, it was possible in this study, unlike in the previous one, to ask whether the mucosal activity patterns elicited by the odorants also varied in a parallel orderly fashion. That is, do the patterns developed on the mucosa reflect the molecular structure of the odorants in some systematic way? The results of this study strongly indicate that, at least for the homologous series of aldehydes used, there is a concordance between an odorant's molecular structure and the mucosal activity pattern it elicits.

In addition to further emphasizing the parallel between the neurophysiologic and behavioral odorant spaces (Figs. 7 and 9), the similar reversal points in both spaces as chain length increases gives rise to yet another speculation. Since on the *x*-axes the reversals occur at C7/8 and on the *y*-axes there are shallower reversals at C9, one might speculate that each dimension represents a different receptor type which is tuned to different aldehydes with one receptor type represented on the *x*-axes and the other one on the *y*-axes. One might further consider the possibility that there is a parallel between the findings of this study and what is known about another aldehyde sensitive receptor, namely I7 [33]. That is, I7 responded best to aldehydes with a U-shaped response profile with its maximum sensitivity at C8, after which sensitivity falls off as chain length continues to increase or decrease. However, at present all this is, of course, merely intriguing speculation.

In the Introduction to this paper it was emphasized that although mucosal activity patterns have been reported in many papers over many years, there has been a dearth of studies actually relating them to olfactory perception. As proposed in the Introduction, an initial step to test whether mucosal activity patterns could play a role in olfactory perception would be to determine whether olfactory perception (as measured by an animal's ability to differentiate between different odorants) is systematically related to these neurophysiologically recorded mucosal activity patterns. A negative result would severely discredit the concept that mucosal activity patterns underlie olfactory quality perception and would strongly argue against further pursuing that possibility. However, the present study did show a significant relationship between the perceptual differences among odorants and the differences in the patterns of mucosal activity they produce. This gives added credence to the concept that the latter may underlie the former.

This added credence, however, does not say that the mucosal activity patterns are an indisputable step in the chain of events leading to perception. It is possible that the spatial patterns are themselves a reflection of the specific molecular response range of the receptors which are differentially distributed on the mucosal surface in band-like distributions. These, in turn, would adequately represent the ligands of different odorants by signaling their unique ligand specificities without any additional contribution from the spatially based mucosal activity patterns. Therefore, a more critical test of the contribution to odorant perception by the mucosal spatial activity patterns would be to devise a method, if possible, for only altering a given odorant's mucosal activity pattern and then test whether the perception of its odor is changed. At this point, however, the results of the present study certainly suggest that the mucosal activity patterns cannot yet be ruled as a possible neural basis for differential odorant perception and must accordingly be maintained as an open and viable option.

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