Title Page

Title: OMP Gene Deletion Results in an Alteration in Odorant-Induced Mucosal Activity Patterns

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Abr. Title: OMP Gene Deletion and Inherent Activity Patterns

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Abstract

Previous behavioral work, using a complex five-odorant identification task, demonstrated that olfactory marker protein (OMP) is critically involved in odor processing to the extent that its loss results in an alteration in odorant quality perception. Exactly how the lack of OMP exerts its influence on the perception of odorant quality is unknown. However, there is considerable neurophysiological evidence that different odorants produce different spatiotemporal patterns of neural activity at the level of the mucosa and that these patterns predict the psychophysically determined perceptual relationship among odorants. In this respect, OMP gene deletion is known to result in a constellation of physiologic defects (i.e., marked reduction in the EOG, and altered response and recovery kinetics) that would be expected to alter the odorant-induced spatiotemporal activity patterns that are characteristic of different odorants. This, in turn, would be expected to alter the spatiotemporal patterning of information that results from the mucosal projection onto the bulb, thereby changing odorant quality perception. To test the hypothesis that odorant-induced mucosal activity patterns are altered in mice lacking the gene for OMP, we optically recorded the fluorescent changes in response to odorant stimulation from both the septum and turbinates of both OMP-null and control mice, using a voltage-sensitive dye (Di-4-Anepps) and a Dalsa 120 x 120, 12 bit CCD camera. To maintain continuity with the previous behavioral work, the odorants 2-propanol, citral, carvone, ethylacetate, and propyl acetate were again used. Each odorant was randomly presented twice to each mucosal surface in a Latin Square design. The results of this study demonstrated that, for both mouse strains, there do indeed exist different spatiotemporal activity patterns for different odorants. More importantly, however,
these patterns significantly differed between OMP-null and control mice. That is, although the general regions of characteristic activity for different odorants were the same in both mouse strains, the patterns in the null animals were degraded relative to controls. These data suggest, therefore, that the alterations in mucosal activity may serve as the substrate for the behaviorally observed changes in odorant quality perception in the null mutant.

Key Words: optical imaging, olfactory marker protein, OMP, sensory transduction, mucosal activity patterns
Introduction

Expression of the olfactory marker protein (OMP) gene is developmentally regulated, phylogenetically conserved, and highly restricted to mature olfactory chemosensory neurons (Margolis, 1980; Buiakova et al., 1994). The presence of this abundant 19kDa cytoplasmic protein in olfactory neurons of vertebrate species as diverse as fish and humans has become a hallmark of the mature olfactory neuron phenotype (Margolis, 1980; Rama Krishna et al., 1992; Buiakova et al., 1994). This constellation of biological properties strongly suggested that OMP plays an important functional role in olfactory chemoreceptor neurons, an hypothesis that has received strong support as a result of recent studies on mice rendered deficient in this protein. Specifically, mice rendered deficient in OMP by targeted gene deletion have a reduced ability to respond to odor stimuli (Buiakova et al., 1996; Ivic et al., 2000). This altered physiological ability was reflected in prolonged onset and recovery kinetics following stimulation and a slower recovery from adaptation. As an additional consequence, the defects in neural activity also resulted in an altered bulbar phenotype that was consistent with apparent abnormal neuronal activity projecting to the bulb (Buiakova et al., 1996), although conduction velocity of olfactory receptor neuron axons was unaltered (Griff et al., 2000). Behaviorally, the observed physiological defects resulted in a behavioral threshold sensitivity that was 50 -100 times less sensitive in knockout animals (Youngentob and Margolis, 1999).

The observed sequela of gene deletion suggested that OMP is an important modulator in olfactory detection/signal transduction processing. However, the functional effects have recently been shown to extend beyond alterations in behavioral sensitivity. Using a complex five-odorant identification confusion matrix task, Youngentob et al. (2001) demonstrated that the untoward physiologic effects associated
with the loss of OMP also resulted in an alteration in odorant quality perception in the null mutant. Exactly how the physiologic defects in mucosal function resulted in altered quality perception is presently a matter of conjecture that requires investigation. In this respect, one aspect of odorant quality coding that warrants consideration is the inherent differential spatiotemporal patterning of neural activity at the level of the olfactory epithelium in response to different odorants (Kauer and Moulton, 1974; Moulton, 1976; Kubie et al., 1980; MacKay-Sim and Kubie, 1981; MacKay-Sim et al., 1982; MacKay-Sim and Shaman, 1984; Kent and Mozell, 1992; MacKay-Sim and Kesteven, 1994; Youngentob et al., 1995; Youngentob and Kent, 1995; Kent et al., 1995, 1996; Scott et al., 1997). These inherent spatiotemporal patterns, which have been shown to predict the behaviorally determined perceptual relationship among odorants (Kent et al., 1995, 2003), likely have as their underlying mechanism the summed effect of: (1) the variations in odorant receptor tuning that exist both within and across the mucosal receptor zones (Malnic et al., 1999); and (2) the clustered expression patterns of some receptor types (Kubick et al., 1997) coupled with the heterogeneous expression patterns of others (Iwema et al., 2003).

If the mucosal representation of an odorant is, indeed, distributed such that large-scale patterns of activation serve as the first step in odorant analysis then the observed physiologic defects associated with OMP gene deletion would be expected to affect odorant quality coding. That is, the altered responsivity of the mucosa coupled with the previously reported slowed response and recovery kinetics might be expected to degrade the odorant-induced spatial activity patterns that are characteristic of different odorants, as well as alter the differential temporal activation of the epithelium. These effects, in turn, would change the organized and stereotyped spatial and temporal patterning of information that occurs at the level of the peripheral projection
from the epithelium to the bulb (Shepherd et al., 1991; Vassar et al., 1994; Mombaerts et al., 1996), thereby altering the perception of odorant quality. Therefore, to test the hypothesis that odorant-induced mucosal activity patterns are altered in mice lacking the gene for OMP, we optically recorded the fluorescent changes in response to odorant stimulation from both the septum and turbinates of OMP-null mice and determined whether null mice differed from age, sex and genotyped-matched controls.

Methods

*Mucosal Preparation*

Ten adult mice (five OMP-nulls on the 129S3/SvImJ background and five 129S3/SvImJ controls) were individually housed and maintained in a temperature and humidity controlled vivarium. Following previously established procedures (Yougentob et al., 1995), mice were anesthetized, decapitated and the extraneous tissue dissected, retaining the region from the naris to the cribriform plate. The right nasal cavity was split into two halves along the length of the nasal cavity, exposing the septum and medial surface of the turbinates (Fig. 1). The two halves of the nasal cavity were placed in an oxygenated Ringer’s solution for 20 minutes after which the preparation was soaked for an additional 20 minutes in the voltage-sensitive dye di-4-ANEPPS. Unbound dye was removed by returning the preparation to Ringer's solution for an additional 20 minutes. Prior to recording from one of the two halves of the right nasal cavity, the tissue was placed in a Delrin chamber and covered with a clear plastic plate (Kent et al., 1996). For the purpose of stimulus delivery (see below), the Delrin chamber had an input and output port designed to be in anatomical register with the external naris and nasopharynx, respectively. Moreover, the chamber was designed to establish rapid onset and clean out of the stimulus while at the same time providing a
uniform flooding of the chamber with an odorant. As such, the chamber permitted the monitoring of the inherent mucosal response (i.e., independent of any imposed chromatographic effect [Mozell and Jagodowicz, 1974]).

**Optics and Electronics**

Except for minor modifications in the optics to accommodate the mouse preparation, and the implementation of a CCD camera (Kent et al., 2003) as the recording device, rather than a photodiode array, the recording methods were identical to those previously described (e.g., Kent and Mozell, 1992; Yougentob et al., 1995) (Fig. 2). Briefly, a real fluorescence image of the mucosa stained with di-4-ANEPPS was projected onto a Dalsa (CA-D1) 12 bit, 120 x 120 digital camera. With the present configuration a 3.5mm x 3.5mm area of the mucosa was imaged onto the camera array with each detector receiving light from a 30μm x 30μm area of the preparation. Data were acquired at a rate of 40 frames per sec.

Consistency in the positioning of the recording surface images on the camera array was accomplished by use of the CCD camera, itself. The digital output of the
camera was viewed directly in order to align each tissue with a standard preparation outline for both the septum and turbinates, respectively. Each tissue was positioned such that particular anatomical landmarks would fall upon specific outline locations. Given the minor anatomical variability that existed from animal to animal, this approach allowed for maximum consistency in alignment across experiments.

**Odorants and Stimulus Delivery**

For each of five odorants (propyl acetate, 2-propanol, citral, l-carvone and ethylacetoacetate), a concentration was chosen such that the size of their respective response were approximately equal and each fell within the dynamic range of their individual concentration-optical response relationship (Youngentob et al., 1995). The concentrations (expressed as percentage vapor saturation @ 23°C) chosen were: propylacetate - 0.75%; 2-propanol - 25%; citral - 45%; l-carvone - 25% and ethylacetoacetate - 13% (N.B.: It should be emphasized that the preceding odorant
stimuli are expressed in terms of the nominal concentrations required to achieving our outlined response parameters under the artificial conditions of our odorant delivery procedure. Based on the physical parameters of the procedure, specifically, the internal volume of the Delrin chamber, the distance between the mucosal surface and the clear plate, the diameter of the input to the chamber, 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 a behaving animal [Kent et al., 2003]). In addition, a sixth odorant, 1% amyl acetate, served as a standard stimulus. Odorant stimuli were generated according to previously established methods, using standard flow dilution olfactometry and computer-driven electronic mass flow controllers (Teledyne Hastings Raydist, Hampton, Va) (Youngentob et al., 1991).

The odorant delivery system was identical to that previously described (Kent et al., 1996). The input port to the Delrin chamber was connected to a “T” connector through which either odorized or deodorized air flowed at a rate of 600cc/min. In order to draw air through the chamber and across the mucosa, a negative pressure ample enough to produce a constant flow rate of 250cc/min was applied to the output port. During stimulation, computer activated valves switched the flow through the T connector from deodorized to odorized air for one second and data were acquired for a total of 45 sec, following initiation of the stimulus pulse.

**Protocol**

The mucosal responses from both the mouse’s septum and medial surface of the turbinates were recorded in each experimental session. The order of tissue recordings was randomly determined. For each mucosal surface, a recording session consisted of a
single presentation of each of the five odorants. The inter-stimulus interval between odorant presentations was approximately five minutes. This interval was chosen in order to ensure complete recovery of the mucosal response to odorant in the null mutant (Buiakova et al., 1996; Ivic et al., 2000). The order of each odorant presentation was determined using a randomized block Latin Square design. This design was done in order to minimize any bias from the order of odorant presentation across animals. In addition, the standard stimulus, amyl acetate, was presented at the beginning of a session, and following the fifth stimulus. The purpose of the standard was to adjust for any gradual changes in mucosal responsivity over time (Youngentob et al., 1995). These shifts in responsivity were independent of changes in baseline drift over time (which was due to photo-bleaching of the dye) or of variations in background fluorescence on a diode-by-diode basis. Correction of the raw responses for baseline drift and level of background fluorescence were accomplished, following previously established procedures (Kent and Mozell, 1992).

For each stimulus presentation and each of the 14,400 contiguous pixels of the camera’s array, the background fluorescence, the average height of the response and the latency of the response (i.e., both start and peak times) were calculated. As previously described (Kent and Mozell, 1992), the start of the response was defined to occur when the rising phase of the response was equal to $1/e$ of the peak response.

Results

*Characterization of the neuronal response in the two mouse strains.*

Previous studies in both amphibians (Kent and Mozell, 1992) and rodents (e.g. Youngentob et al., 1995; Kent et al., 1996) have demonstrated that the optically recorded response to odorant stimulation has a similar profile and response characteristics as
those of the EOG (Ottoson, 1956). Thus, by analogy to the EOG the optically recorded response monitors a component of the generator potential of sensory neurons responding to odorant stimulation. Nonetheless, in considering the following results it should be recognized that the two techniques differ in a number of ways. Specifically, the present optical recording procedure monitors the dc-coupled voltage change (Kent et al., 2003) which predominantly, if not exclusively, originates from the cilia in response to odorant stimulation (Kent and Mozell, 1992; Yougentob et al., 1995). By contrast, the standard EOG is an ac-coupled signal (e.g., Buiakova et al., 1996; Ivic et al., 2000) which monitors current flow through a number of cellular elements within the epithelium (Scott and Scott-Johnson, 2002).

As examples of the raw responses, Figure 3 shows one array for each mouse strain (i.e., both OMP-null and 129S3/SvImJ controls) in response to the odorant propanol delivered to the turbinate mucosa. The optically recorded response traces are shown for every fourth pixel of the 120x120 array. The response tracings, in turn, are superimposed upon a color-scale representation of average response magnitude for all pixels of the camera array. The larger the response the brighter the pixel. As can be seen in Figure 3 (insert A), the response of the mucosa was a typical slow monophasic potential. More importantly, however, although propanol gave responses across the entire sampled area of the two mucosas, the responsivity of the two mouse strains appears qualitatively different. Specifically, focusing on the brightness across the two panels of Figure 3 gives the general impression that the response profile of the OMP-null animal was more subdued or muted relative to controls.

Figure 4 represents the raw average pseudo-color topographical response profiles for the 120x120 array in response to each of the five odorants. In keeping with the above, although the average raw patterns for each odorant were similar between
control and null animals, (i.e., region of maximal differential activity) a change in responsivity across the epithelium between the two mouse strains can be appreciated in the fine detail. For example, focusing on the center of each mucosal response panel for the odorant propanol, compared to controls, the response of the null animal was more evenly distributed. That is, the gradient of differential activity across the epithelium was reduced or muted.

In extension to the above, Figure 5 highlights the effect of OMP gene deletion on the raw responses of the epithelium. For each of three parameters of the optically recorded response (i.e., average height, start time, and peak time) the panels of Figure 5
were determined by calculating, pixel-by-pixel, the ratio of the raw control response divided by the raw response of the null animal. Thus, for a ratio value >1 the response of the control animals, at any given pixel, were greater than the nulls and vice versa. As illustrated for the turbinate response to odorant stimulation, for those regions of the epithelium where the responsivity to a particular odorant was maximal (see Figure 4) the control animals gave a relatively larger response, as compared to the nulls. By contrast, for those areas of the epithelium where the control animals were least responsive to a particular odorant the null animals gave a relatively larger response. As emphasized by the scale bar provided in Figure 5, these noted differences were rather robust across the epithelium. For example, the ratios for the average response
magnitude represent a 2.0, 2.1, 2.3, 2.3 and 2.3 fold change in mucosal response for the
odorants carvone, citral, ethylacetooacetate, propyl acetate and propanol, respectively.
In total, these shifts resulted in similar but muted raw response patterns for the null
mutant (as seen in Figure 4). Comparable results were observed for the septal mucosa
as well.

Figure 5 also illustrates a change in raw temporal activity between mouse strains.
That is, where the spatial pattern for the control animals was larger than the nulls, the
response latency (i.e., start time) and time to peak response was also relatively faster. In
contrast, for those areas of the epithelium where the control animals were least
responsive to odorant stimulation the response latency and time to peak response of the
null animals was relatively faster. Thus, both changes in responsivity and kinetics were
observed between mouse strains. However, in extension to previous work using EOG
recording from a markedly smaller sample of the mucosa (Buiakova et al., 1996; Ivic et
al., 2000) the alterations observed in the present study were location dependent.
Nonetheless, taken together, these location dependent changes resulted in a more
broadly distributed and degraded raw response in the null animals.

Spatial activity patterns as a function of odorant and genotype

In summary of the above, the raw response data demonstrated that the mucosal
response to odorant stimulation varied both as a function of odorant and mouse strain.
As previously noted, these spatiotemporal patterns of activity likely result from the
collective effect of the variations in odorant receptor tuning that exist among the
intermingled receptor types both within and across receptor zones, the clustered
expression patterns of some receptor types and the heterogeneous expression patterns
of others. Therefore, to remove common elements of broad responsivity and highlight
the areas of differential odorant activity for each of the two mouse strains in response to each of the five odorants, the following procedure was used. For each animal and each presentation of odorant the responses monitored by each pixel were equilibrated (i.e., scaled) to unity, according to previously established methods (Kent and Mozell, 1992). This equilibration process removed any differences in response magnitude between arrays so that magnitude differences would not be confused with pattern differences. For each odorant and each mouse strain, the equilibrated average response size was then averaged across animals on a pixel-by-pixel basis. A summary map of differential spatial activity for a given odorant and mouse strain was then produced by subtracting,
pixel-by-pixel, the average height of the response in a given pixel by that same pixel’s average response across all five odorants (Fig. 6). The differential response at each pixel was represented using a pseudo-color scale (Kent et al., 2003). As can be seen in this figure, and in keeping with prior work in other species, each odorant produced a unique region of increased responsivity that distinguished it from every other odorant. In this regard, although there were indeed minor variations in the shape of increased activity for each of the odorants, the unique geographic region was, for all animals in a given strain, in a qualitatively similar area of the mucosa (data not shown). In addition, the general area of increased activity appeared similar across both mouse strains, suggesting that the geographic components of the differential spatial response patterns are the same in control and null animals. Nonetheless, the data in Figure 6 also give the impression that the two mouse strains differ with respect to the magnitude of their unique differential odorant response patterns. That is, for each of the odorants tested there was an increasing trend toward equal responsivity across the epithelium in the null animal. Thus, for example, focusing on the response to ethylacetooacetate, the region of relative maximal activity along the dorsal surface is dramatically reduced in the null animal.

To further emphasize the apparent muting of responsivity between the two mouse strains, the data developed for the construction of Figure 6 were again used. However, for the purpose of Figure 7, only those pixels which demonstrated relatively greater activity were considered (those that showed decreased sensitivity relative to the average were set to 0). These data were then transformed into enhanced color scale surface plots such that the magnitude of the difference of increased relative responsivity for each pixel of the 120x120 array was represented by the height on the z-axis. As emphasized in the panels of Figure 7, each odorant produced a unique area of
differential activity on the septal mucosa of both mouse strains that distinguished it from every other odorant. More importantly, however, relative to control animals (Fig. 7: top row), the differential spatial response to an odorant appears to be degraded or muted in the null mutant (Fig. 7: bottom row). Similar results were observed for the turbinate mucosa as well.

As emphasized in Figures 6 and 7, the general physical pattern of differential spatial activity in response to different odorants appear to be generally similar in both the OMP-null and control animals, while the distinctiveness of the patterns appeared altered as a function of genotype. To formally evaluate these observations we first examined whether the five odorants, do indeed, produce differential patterns of
response and whether these patterns varied as a function of genotype. In order to accomplish this the following procedure was used. First, for the present and all subsequent analyses, the septal and turbinate mucosas were considered as one complete expanse of tissue. Second, for each animal and each presentation of odorant, the responses monitored by each pixel were equilibrated, as previously noted above. Third, the differential spatial activity pattern for a given odorant, in a given animal, was produced by subtracting, pixel-by-pixel, the average height of the response in a given pixel by that same pixel’s average response across all five odorants. Finally, the fourth step was to calculate the degree of dissimilarity between the differential response arrays for each of the five odorants tested both within an animal and between every possible pair of animals (i.e., both OMP-nulls and controls). The degree of dissimilarity between any two differential response arrays was calculated by summing the absolute values, on a pixel-by-pixel basis, of the difference between the two paired response arrays, and then dividing that summation by the total number of pixels in the array. As a result, this measure served as an indication of the degree of response dissimilarity both as a function of odorant presented and genotype. These comparisons, in turn, yielded a 50x50 dissimilarity matrix for the entire sampled mucosal surface. The 50x50 dissimilarity matrix was subjected to multi-dimensional scaling analysis (MDS) (Schiffman et al., 1984), with the objective of placing an animal’s response to the presentation of an odorant in an “odorant/mouse space” according to the degree of dissimilarity in odorant-induced spatial activity patterns (Loo et al., 1996). The MDS analysis yielded a two-dimensional solution, thereby providing a set of coordinates which located each mouse’s (both OMP-null and control) response to a particular odorant within the MDS space. To evaluate formally whether the odorant-induced spatial activity patterns varied as a function of odorant and/or genotype, a multivariate
analysis of variance (MANOVA) was performed, using the MDS coordinates as the dependent variables and, odorant and genotype as the independent variables. The results of these analyses demonstrated highly significant effects of odorants ($F_{8, 86} = 9.604; P = \text{nil}$), thereby giving further support to the existence of different spatial activity patterns across the mucosa for different odorants. In contrast, however, there was no effect of genotype ($F_{2,43} = 0.0017; P = 0.998$), suggesting consistency in the location of the observed patterns of greater and lesser relative responsivity for each odorant across the two mouse strains. Taken together, these results confirmed our subjective impression that while different odorants produced different inherent activity patterns across the two mucosal surfaces sampled, these patterns were similar in the two mouse strains.

To evaluate the apparent degrading or muting of the spatial response pattern in the null animals (Fig. 7), the distinctiveness of an odorant’s differential mucosal response was quantitatively expressed as the average percentage difference (APD) (Kent and Mozell, 1992; Loo et al., 1996). For each of the animals (both OMP-null and control), the APD for a given odorant was calculated by subtracting, pixel-by-pixel, the equilibrated individual odorant pattern of interest by the equilibrated average response pattern of all five odorants; calculating the sum of the absolute value of the differences across the pixels; dividing by the number of contributing pixels; and multiplying by 100. This APD expression, therefore, quantified the average, pixel-by-pixel, percentage difference between the odorant of interest and the average of all five odorants in a particular animal. As can be seen in Table 1, for each of the five odorants the APD decreased markedly as a function of genotype. On average, the patterns of differential
responsivity were 23.5% less distinct or muted in the null mutant with the response to the odorant propyl acetate showing the least muting (i.e., 9.95%) and that to propanol showing the greatest (i.e., 34.67%).

To test formally the significance of the apparent genotype related shifts, we evaluated the null hypothesis that the distinctiveness of the odorant patterns, as expressed by the APD, did not change with genotype. The data from the individual animals were subjected to an analysis of variance, with the principle analysis of interest being the effect of genotype on the APD. The results of this analysis gave strong support to the hypothesis that a muting or degradation of the odorant-induced spatial patterns occurred as a function of OMP gene deletion ($F_{1,44}=12.313; P = 0.0011$).

**Table I. Distinctiveness of spatial activity patterns varied with genotype**

<table>
<thead>
<tr>
<th></th>
<th>Carvone</th>
<th>Citral</th>
<th>Ethyl acetoacetate</th>
<th>Propyl acetate</th>
<th>Propanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26.93</td>
<td>23.13</td>
<td>22.89</td>
<td>9.95</td>
<td>34.67</td>
</tr>
</tbody>
</table>

The % change in APD is reported for each odorant. Each entry represents the difference between the average APD for control and OMP-null animals. The APD was calculated as described in the text.

Temporal activity patterns as a function of odorant and genotype

In addition to differing spatial patterns of mucosal activity for different odorants, there also existed differing temporal characteristics of the responses. We first
evaluated, using the procedures outlined above, whether the five odorants produced different temporal patterns of response latency (i.e., start time), and whether these temporal patterns varied as a function of genotype. Second, using our APD measure, we evaluated the apparent muting of the temporal response pattern as a function of OMP gene deletion. The results of these analyses demonstrated: (1) a highly significant effect of odorant ($F_{8,86} = 5.548; P = \text{nil}$) and no effect of genotype ($F_{2,43} = 0.317; P = 0.968$) on determining the differential temporal odorant response of the epithelium; and (2) a significant effect of genotype on the APD of the temporal response pattern ($F_{1,44} = 16.318; P = 0.0002$). With regard to the later formal result, Table 2 illustrates the

<table>
<thead>
<tr>
<th>Carvone</th>
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<th>Ethyl acetoacetate</th>
<th>Propyl acetate</th>
<th>Propanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.64</td>
<td>26.71</td>
<td>16.47</td>
<td>28.50</td>
<td>33.99</td>
</tr>
</tbody>
</table>

The % change in APD is reported for each odorant. Each entry represents the difference between the average APD for control and OMP-null animals. The APD was calculated as described in the text.

Insert Table 2. About Here

change in average APD data as a function of genotype. For each evaluated odorant the APD decreased in the null animal. On average, the distinctiveness of the temporal response pattern decreased 26.66% in the null mutant with the smallest change attributable to the ethylacetoacetate response (16.47%) and the largest change occurring in the propanol comparison. Therefore, as a whole, the data suggest that while different
odorants produced different temporal patterns of activity across the epithelium, these temporal patterns were similar in the two mouse strains for any given odorant. Nonetheless, the results support the supposition that a muting or degradation of the temporal activation pattern occurred as a function of OMP gene deletion.

Discussion

Prior behavioral evaluations, using a five odorant identification confusion matrix task, demonstrated that OMP is critically involved in odor processing to the extent that its loss resulted in an alteration in odorant quality perception (Youngentob et al., 2001). This observation coupled with the known neurophysiological defects at the level of the epithelium (Buiakova et al., 1996; Ivic et al., 2000) led to the suggestion that one possible mechanism underlying the change in quality perception in OMP-null mice might be an alteration in the spatiotemporal response to odorants across the mucosa. Thus, the major objective of the present investigation was to evaluate whether, and to what degree, the large-scale spatiotemporal response of the epithelium to different odorants was altered, relative to controls, in the null mutant.

With regard to the above proposition, the qualitative and quantitative data of the present study addressed two fundamental issues relevant to the spatiotemporal differentiation of odorants in the two mouse strains. Specifically, we evaluated: (1) whether the odorant-induced spatiotemporal activity patterns varied as a function of odorant and/or genotype; and (2) whether the distinctiveness of the differential patterns were muted or degraded as a function of OMP gene deletion.

In addressing Question 1, the present study confirmed for both mouse strains the existence of different spatiotemporal activity patterns in response to different odorants. As such, the data extend to yet another species the prior observations obtained in both
rats (MacKay-Sim and Kesteven, 1994; Youngentob et al., 1995; Youngentob and Kent, 1995; Kent et al., 1995, 1996; Scott et al., 1997; Kent et al., 2003) and two amphibian species (Kauer and Moulton, 1974; Moulton, 1976; Kubie et al., 1980; MacKay-Sim and Kubie, 1981; MacKay-Sim et al., 1982; MacKay-Sim and Shaman, 1984; Kent and Mozell, 1992) that non-homogeneous patterns of sensitivity to different odorants exist along the olfactory epithelium. In addition, the application of the CCD camera to the optical recording technique (with its 14,400 pixels) provided a more fine-grained method (over the previous 10 X 10 photodiode array [e.g., Kent et al., 1992; Youngentob et al., 1995; Kent et. al., 1996) for the evaluation of the epithelial response to odorants. In this regard, a secondary observation in this study was a band-like configurations of mucosal activity patterns (in both mouse strains) reminiscent of the zonal distribution of odorant receptors (Dear et al., 1991; Nef et al., 1992; Ressler et al., 1993; Strotmann et al., 1992; Vassar et al., 1993). This finding was consistent with those of Kent et al. (2003) (recording from rats) who emphasized that while the neurophysiologic data appeared consistent with the conclusion that receptors are arranged in zones, they were not consistent with the notion that like receptor types are necessarily distributed homogeneously within a zone. Clearly, in the present study, the response areas both within and across reported receptor zones (Fig. 4-raw responses; Fig. 6-differential odorant pattern responses) are far from homogeneous. This lack of homogeneity in responsivity was consistent with the observations of several molecular studies that: (1) some receptor types (e.g., OR37 and OR-Z6) have a clustered distribution (Breer et al., 1994; Strotmann et al., 1994; Kubick et al., 1997; Pyrski et al., 2001); and (2) odorant receptors previously held to be randomly distributed within a given zone are instead distributed heterogeneously with an aggregation of receptors in a particular region (Iwema et al., 2003).
With regard to the principal focus of the study, the results clearly demonstrated that the epithelium of OMP-null mutants responded to odorant stimuli with both spatial and temporal activity patterns that resembled the patterns observed in control animals. That is, as seen in both the raw responses illustrated in Figure 4, the differential responses of Figures 6 and 7, and confirmed quantitatively, the relative areas of spatiotemporal activity for each odorant did not appreciably shift to different locations within the sampled area as a function of OMP gene status. Accordingly, this component of the neurophysiological results suggests that, at least at the level of the differential organization of the odorant receptors both within and across receptor zones (Dear et al., 1991; Iwema et al., 2003; Kubick et al., 1997; Nef et al., 1992; Ressler et al., 1993; Vassar et al., 1993), odorant coding as a function of the differential response of different receptors (Malnic et al., 1999) appears well maintained despite the loss of OMP.

The quantitative and qualitative results of Question 2 gave strong support to the hypothesis that while the general spatiotemporal patterns of activity did not shift as a function of gene deletion, the patterns became muted or degraded (Figs. 4, 6, and 7). In other words, for both the spatial and temporal response to an odorant, there was a significant increase toward equal responsivity across the epithelium in the null mutant. On average, the raw spatial and temporal response patterns were, relative to controls, degraded by a factor of 2.2-fold. Moreover, when the distinctiveness of the differential spatial and temporal mucosal response to different odorants was emphasized the responses of the null animals were muted by 23.5% and 26.66%, respectively. In considering the implications of these results, the dichotomy between the distinctiveness data and the pattern results requires some comment. Specifically, one might question how the muting or degrading of the pattern would not manifest itself in an alteration of
pattern, per se. The answer to this question lies in the analyses themselves. On the one hand, using our measure of pattern dissimilarity, the MDS analysis focused, on a pixel-by-pixel basis, on the physical location of differential mucosal activity in response to the various odorants. By comparison, recall that the APD measured the absolute differences between an individual odorant pattern of interest and the average pattern of all five odorants, without regard to exactly where on the epithelium the area of differential activity occurred. That is, for any comparison, we calculated, on a pixel-by-pixel basis, the absolute difference in equilibrated responses between corresponding pixels of the paired arrays. Consequently, only in the case where a change in the characteristic maximal area of differential odorant responsivity occurred (i.e., equal responsivity relative to the average) would we expect to see a “shift” in the pattern coincide with an apparent muting.

In summary of the above, response differences between mouse strains were clearly observed in the raw data illustrated in Figures 4 and 5, as well as in the differential odorant response data of Figures 6 and 7. Further, quantitative analysis demonstrated that the underlying differential patterns of response to different odorants were not altered (i.e., whole-sale shifts in spatial location) between mouse strains, but rather, the patterns were muted or degraded (i.e., more broadly distributed) in the null mutant. Whether the later observation, in fact, constitutes a “shift” or difference in differential odorant response pattern, per se, is partly philosophical. Nonetheless, taken together the data demonstrate an alteration in the epithelial response to different odorants in the OMP-null animal.

The two sets of results (i.e., similar but degraded patterns) were not entirely surprising given the present working hypothesis regarding OMP’s role in odor processing. The current body of evidence suggests that OMP plays a novel modulatory
role in the odor detection/signal transduction process (Buiakova et al., 1996; Ivic et al., 2000; Margolis et al., 1997; Yougentob and Margolis, 1999). Neurophysiologically, loss of the protein has previously been shown by others to result in a reduction in peak response magnitude in paired-pulse experiments, and a slowing of onset and recovery kinetics, as measured by the EOG (Buiakova et al., 1996; Ivic et al., 2000). Thus, a priori, there was no expectation that the lack of OMP would alter the stereotyped expression pattern of odorant receptors across the olfactory epithelium (Dear et al., 1991; Nef et al., 1992; Ressler et al., 1993; Strotmann et al., 1992; Vassar et al., 1993; Kubicket al., 1997; Iwema et al., 2003). Indeed, the results outlined above confirm this proposition. Rather, the present results are in keeping with the notion that gene deletion alters the responsivity of the epithelium to an odorant as well as the kinetics of the response. However, in extension to prior observations (Buiakova et al., 1996; Ivic et al., 2000) the present data (under our setting of monitoring large-scale patterns of activation) would further suggest that the relative effect of gene deletion on the differential response of the epithelium to an odorant was location dependent. This, in turn, resulted in a significant increase toward equal responsivity across the epithelium (Figs. 4 and 5).

Based on the foregoing, it is worth considering how the data obtained in the present study relate specifically to those of previous investigators (Buiakova et al. 1996; Ivic et al., 2000). As noted, prior studies could be interpreted as suggesting that an overall change in both the time-course and magnitude of the odorant response occurs in the null animal, whereas the current study suggests that these alterations, relative to controls, varied with location. In this regard, there are a number of methodologic differences that could account for this disparity. First, by contrast to previous studies using the EOG, which sampled a very limited punctate area of the epithelium, the
present study applied optical recording methods (in conjunction with a voltage-
sensitive dye) to study the large-scale activation patterns across the extent of the
epithelium in a fine spatial matrix. Therefore, the current method, by providing a more
comprehensive and accurate representation of the mucosal response to an odorant in
both space and time may have been more sensitive to potential variations in the
epithelial response of the OMP-null animal. Second, the stimulation parameters used in
the current study contrast sharply with those previously used. As noted in the
Methods, the inter-stimulus interval between odorant presentations was approximately
five minutes. Based on the prior EOG studies (Buiakova et al., 1996; Ivic et al., 2000) this
interval was chosen in order to ensure complete recovery of the mucosal response to
odorant stimulation in null animals. Thus, while the earlier work emphasized
stimulation paradigms (i.e., paired-pulse stimulation and short inter-stimulus intervals)
designed to exacerbate the consequences of gene deletion on the neuronal response the
current study was directed toward examining the large-scale spatial response patterns
under unperturbed conditions. As such, the stimulus conditions of the current study
may have been more appropriate to uncover subtle response differences across the
extent of the epithelium. Finally, there are distinct technical differences between the
EOG and optical recording techniques. Although both methods have similar profiles
and response characteristics the two likely monitor different components of the
generator potential. That is, the present optical recording procedure monitored the dc-
coupled voltage change of the epithelium in response to odorant stimulation.
Moreover, this response predominantly, if not exclusively, originated from the cilia. By
contrast, the EOG method applied in prior studies on OMP-null animals was a low
frequency filtered ac-coupled signal that monitored current flow through a number of
cellular elements within the epithelium. Therefore, it is highly likely that the two
methods monitored correlated yet different components of the neural response potentially relating to the differences observed in the current study.

Given the present findings, it is important to consider exactly how a muting of the spatial and temporal response of the epithelium might translate into an alteration in odorant quality perception. At the level of the olfactory bulb there is ample evidence for both a spatial and temporal component to the encoding of odorant quality (Chaput et al., 1992; Cinelli et al., 1995; Freidrich and Korsching, 1997; Imamura et al., 1992; Johnson et al., 1998, 1999, 2000; Kauer and Cinelli, 1993; Laurent et al., 1996; Laurent, 1999; Macrides and Chorover, 1972). We propose, therefore, that the alterations in neural function at the periphery were translated to the bulb. In terms of spatial coding, it has been suggested that the bulb is an array of functional modules (i.e., clusters of glomeruli) that serve to spatially segregate and tune responses to the molecular features of an odorant (e.g., Johnson et al., 1998, 1999; Johnson and Leon, 2000). The information carried by individual glomeruli, therefore, are a reflection of the periphery by virtue of the organized and stereotyped patterning of information that occurs from the epithelial projection to the bulb (Shepherd, 1991; Vassar et al. 1994; Mombaerts et al. 1996). Consequently, it is reasonable to consider that the degrading of peripheral information would be translated to the bulb. That is, for the active glomeruli in response to an odorant there would be a significant increase toward equal responsivity like that seen on the mucosa, thereby altering the code. In addition, the spatial muting effect might further impact the coding process by interfering with the lateral inhibitory mechanisms which subsequently fine tune the response of mitral and tufted cells to odorant features. Finally, one could further envision how a muting of the temporal sequence of odorant activation would also effect the reshaping of odor codes, resulting from the internal connectivity of early olfactory circuits. In total, therefore, the degraded or disrupted
information at the level of the bulb would be expected to alter the perception of odorant quality.
Acknowledgements

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References


Kubie JL, MacKay-Sim A, and Moulton DG. Inherent spatial patterning of response to odorants in the salamander olfactory epithelium. In: H. Van der Starr (Ed.), *Olfaction and...*


Scott JW, Scott-Johnson PE. The electroolfactogram: a review of its history and uses.


Figure Legends

Figure 1. Typical mouse mucosal recording preparation. The boxes indicate the approximate recording area for each animal. The opposing septum and turbinates were brought into register for by rotating the septal image around its horizontal axis. CRIB, ANT, NP refer to cribriform, anterior and nasopharynx, respectively. The orientations of all subsequent figures are the same as in Figure 1.

Figure 2. Schematic drawing of the apparatus used to record signals of a 120x120 array. Light from a 24 V 250 W tungsten-halogen lamp was collimated, and the infrared wavelengths filtered with a KG-3 Schott heat filter. The light was made quasi-monochromatic with a 530 nm (65 nm half-bandwidth) excitation filter and focused on the mouse epithelium with a 35 mm f1.4 Nikkor lens. The emitted fluorescence wavelengths from the epithelium were transmitted through a dichroic mirror and 650 nm (100 nm half-bandwidth) emission filter. An image was formed at the focal plan of the CCD camera with a series of lenses.

Figure 3. Optical signals recorded from the turbinate mucosa of a control and OMP-null mouse in response to the odorant propanol. The response traces are shown for every fourth pixel of the 120x120 array. The total length of each tracing is 45 sec. (i.e., the total period over which data were acquired following stimulus onset) 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 14,400 pixels. Thus, the greater the magnitude of the response the brighter the pixel. Panel A illustrates a magnified view of the pixel indicated by the red dot in the control animal array. The vertical height of the red arrow in the panel represents 0.25% change in fluorescence and the horizontal
arrow indicates 20 sec. 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 0.0% change in fluorescence and bright blue to 0.5%. The anatomical orientation is the same as in Figure 1.

Figure 4. Normalized composite raw response patterns for each mucosal surface and each mouse strain in response to, L- carvone (CAR), citral (CIT), ethyl acetoacetate (EA), propyl acetate (PA) and propanol (PRO), respectively. As illustrated by the color scale, for any given pixel of the 120x120 array, violet indicates a response 5% of the maximum response whereas red denotes a relative response equal to 95%. The anatomical orientation is the same as in Figure 1.

Figure 5. For each of three response parameters (AVG: average height, START: latency of the response, and PEAK: time to peak response) the panels illustrate the pixel-by-pixel division of the raw average response of the control animals divided by that of the OMP-nulls. As illustrated by the color scale, violet indicates the control response for a given pixel was 0.6 times that of the null response, whereas red indicates that the control response was 1.4 times the null. Note that a ratio of 1 indicates no difference between the two mouse strains. The odorants are the same as in Figure 4.

Figure 6. Composite spatial response patterns for each mouse strain and both mucosal surfaces (i.e., septum and turbinates) in response to L- carvone (CAR), citral (CIT), ethyl acetoacetate (EA), propyl acetate (PA) and propanol (PRO). As illustrated by the color scale, violet indicates a 15% relative decrease from the average response whereas red denotes a 15% relative increase. The anatomical orientation is the same as in Figure 1.
Figure 7. Composite color scale enhanced surface plots for the septal response of both control and OMP-null animals in response to the five odorants (L-carvone = CAR; citral = CIT; ethyl acetoacetate = EA; propyl acetate = PA; and propanol = PRO). The z-axis corresponds to a change in response for a given odorant at a given pixel compared to the average of all odorants for that pixel after all 120x120 array of responses have been equilibrated to unity. For each panel, the first level, green, was assigned the smallest difference (i.e., zero) and yellow the largest difference (i.e., 15%). The color bar represents the continuum of response between the endpoints. The anatomical orientation is the same as in Figure 1. See text for details.