Comparison of Identified Mitral and Tufted Cells in Freely Breathing Rats: II. Odor-Evoked Responses

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Abstract

Mitral and tufted cells are the 2 types of output neurons of the main olfactory bulb. They are located in distinct layers, have distinct projection patterns of their dendrites and axons, and likely have distinct relationships with the intrabulbar inhibitory circuits. They could thus be functionally distinct and process different aspects of olfactory information. To examine this possibility, we compared the odor-evoked responses of identified single units recorded in the mitral cell layer (MCL units), in the core of the external plexiform layer (not at the glomerular border tufted cells), or at the glomerular border of this layer (GB tufted cells) of the entire olfactory bulb. Differences between mitral and tufted cells were observed only when subtle aspects of the responses were explored, such as the firing rate per respiratory cycle or the distribution of firing activity along the respiratory cycle. By contrast, more clear differences were found when the 2 subtypes of tufted cells were examined separately. GB units were significantly more responsive, had significantly higher firing activity, and showed greater activity at the transition between inspiration and expiration. The projection-type tufted cells situated closer to the entrance of the olfactory bulb may thus form a distinct physiological class of output neurons and differ from mitral cells and other tufted cells in the manner of processing olfactory information.

Key words: main olfactory bulb, mitral cells, odor-evoked responses, olfaction, respiration cycle, tufted cells

Introduction

The vertebrate retina is a model system for sensory processing and the functions of several retinal circuits are fairly well understood (e.g., Dowling 1987; Masland 2001; Wassle 2004). Retinal output via the axons of different classes of ganglion cells conveys different aspects of visual stimuli to the brain (e.g., Enroth-Cugell and Robson 1966; Shapley and Perry 1986; Field and Chichilnisky 2007). On the other hand, the functional roles of mitral and tufted cells, the 2 classes of output neurons from the main olfactory bulb (MOB), are less well understood. In the MOB, the information carried by the axons of nearly 25×10^6 mature olfactory receptor neurons converge upon 2500 glomeruli (Meisami 1991; Meisami and Sendera 1993; Paternostro and Meisami 1996) before being redistributed to the dendrites of about 45 000 mitral cells situated in the mitral cell layer (MCL) and 100 000 tufted cells situated in the external plexiform layer (EPL). Olfactory information is further processed by glomerular and interglomerular circuits in the glomerular layer along with granule cell interactions in the internal layer and EPL. Because several thousand olfactory receptor neurons converge onto a single mitral or tufted cell, the signals carried by the axons of these output neurons of the MOB represent likely the odor information in its most parsimonious form. The goal of this study was to compare and contrast the responses of mitral and tufted cells to odor stimulation. Moreover, by reconstructing electrode tracks, we identified projection tufted cells at the glomerular border (GB units) of the EPL and compared their response properties with mitral and deeper tufted (not at the GB [notGB]) cells. NotGB and GB units were found to differ not only regarding their spontaneous activity (the companion paper) but also in their responses to odors.

As described in more detail in the companion paper (Griff et al. 2008), mitral and tufted cells differ in the location of their somas and in the extent and laminar distribution of their secondary dendrites, and their axons project to

different but overlapping regions of the central nervous system. Recent experiments by Nagayama et al. (2004) indicate that the response of tufted cells to odorants is more robust compared with mitral cells but that tufted cells do not exhibit as much lateral inhibition when presented with a range of olfactory stimuli. These recordings were from a restricted region of the dorsal MOB, which is particularly responsive to straight chain acid odorants.

The present study compares the responses to odor stimulation of mitral and tufted cells located throughout the MOB. All the cells, including those at the GB, were antidromically activated by stimulation of the lateral olfactory track (LOT), confirming their identification as output neurons. Mitral cells were distinguished from tufted cells on the basis of the waveform of the LOT-evoked field potential and from electrode track reconstructions. The present study is distinctive in that responses to odorants were analyzed in terms of the respiratory cycle by constructing respiratory cycle–triggered histograms (CTHs). Thus, if an odorant caused an increase in activity during inspiration and a decrease in activity during expiration (a common pattern) but little change in the mean activity, it would be considered a clear response in the present study. Combined with the results of the companion paper (Griff et al. 2008) comparing the impulse conduction and spontaneous activity of mitral and tufted cells, this paper points to a distinct physiological role of superficial, projection-type tufted cells.

Materials and methods

Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) for the care and use of laboratory animals, and all efforts were made to minimize animal suffering and to reduce the number of animals used. Experimental protocols were approved by the Comité d'Expérimentation Animale de l'Université Claude Bernard—Lyon1.

General surgical and electrophysiological techniques are described in the accompanying paper (Griff et al. 2008). Units were shown to be output neurons based on antidromic activation from the LOT. Based on waveform of the LOT-evoked field potential and electrode track reconstructions, units were identified as mitral (MCL) or tufted (EPL) and further as GB (at the GB) or notGB.

For the duration of the experimental protocol, rats were placed in front of a flow dilution olfactometer; a detailed description of the olfactometer has been published elsewhere (Vigouroux and Chaput 1988). After establishing that the recording was from a single unit and documenting antidromic activation, responses to odor stimulation were recorded. Between odors, the olfactometer continuously delivered and combined 28 l/min of pure air in a main flow with 2 l/min of pure air in a second flow. Odors were produced by replacing 2 l/min of the pure air with 2 l/min of odorized air. This odorized air was obtained by pumping a predetermined amount of saturated vapor from 50l Tedlar bags connected to the olfactometer through preadjusted needle valves. The odorized flow began to be produced 10–15 s before odor delivery to allow the odor concentration to stabilize in the line, and it was exhausted until stimulation onset. Odor delivery was initiated 10 ms after expiration began so that the first inspiration included in the stimulation corresponded to a complete stimulation period. Chemical odors were differentially diluted to obtain a final partial pressure of 2.9 Pa (Giraudet et al. 2002). This concentration corresponded respectively to a dilution of 1.1×10^{-2} and 4.0×10^{-3} of the saturated vapor of cineole (CIN) and isoamyl acetate (ISO) and was considered high enough to recruit most of the olfactory receptor cells responding to the delivered stimulus.

Seven different pure chemical odorants along with rat chow (2 times) were used in this study. They were delivered in the following order: food odor (FO), acetophenon (ACE), CIN, ISO, p-cymen (CYM), methyl-amyl ketone (MAK), anisole (ANI), 2-hexanol (HEX), FO. Each odor presentation lasted 10 s and was separated from the preceding one by at least 1 min. The 30-s period immediately preceding the odor stimulation was taken as the spontaneous activity for that stimulation.

Analyses of spike activity were performed with respect to the respiratory cycle as described in the companion paper (Griff et al. 2008). Spontaneous and odor-evoked activities were characterized by their type and level. Examples of the different CTH types are shown in Figure 6. CTHs were classified as unsynchronized, simple synchronized, or complex synchronized on the basis of the variation of the cell activity during the respiratory cycle. A unit's responsiveness to odorants was determined by comparing each odor-evoked pattern with the corresponding spontaneous (prestimulation) pattern. A positive response was also indicated by an obvious increase or decrease in total number of spikes per respiratory cycle based on examination of the raster displays of spikes during the spontaneous and odor stimulation cycles even if the pattern of the CTH did not change. The number of responses to the various odors was used to compare the responsiveness and reactivity of mitral and tufted cells.

Further analyses were done to determine whether there was a difference between MCL and EPL cells regarding the distribution of their firing activity along the 45 bins of the respiratory cycle, that is, their relationship with respiration. Three variables were utilized to characterize the response CTHs: the maximum, the mean, and the range defined as the difference between the maximum and the minimum values of the CTH. Because the minimum was near zero, it is not reported as a separate variable. Differences between cell types for these variables were assessed by the Wilcoxon, a nonparametric, test.

Discriminant analysis was also utilized to determine whether there was a difference between MCL and EPL cells regarding the distribution of their firing activity along the 45 bins of the respiratory cycle. The goal of the discriminant

analysis was to find the bins that contributed the most to a good discrimination between MCL and EPL units. The method used was a discriminant factorial analysis, performed with the CANDISC and DISCRIM procedures in the SAS software. A discriminant analysis was also performed with spontaneous activity in the companion paper (Griff et al. 2008), and the technique is more completely described there.

Results

In this study, 19 MCL units and 42 EPL units of which 11 were GB and 31 were notGB were recorded from 22 rats. All units were antidromically activated from the LOT and therefore were output neurons from the MOB. Figure 1 presents the response of an MCL and a GB unit to the same odorant recorded in 2 different rats. For each unit, the CTH during stimulation by an odorant was compared with the CTH before stimulation. A change in CTH type or in mean rate indicated a positive response to an odor. There was no significant difference between the mean duration of the respiratory cycle during these odor presentations (Wilcoxon test, 0.05 significance level). Thus, differences between cell types presented below cannot be attributed to differences in cycle duration.

Figure 2A compares EPL and MCL units in terms of their responsiveness to 8 odors (see Materials and methods). Except for food, more that 50% of both the MCL and the EPL units responded to each of the odorants presented. There was a significant difference neither between the 2 presentations of the FO nor between the pattern of responsiveness of MCL and EPL units to the 8 odors or to each odor (Fisher's exact
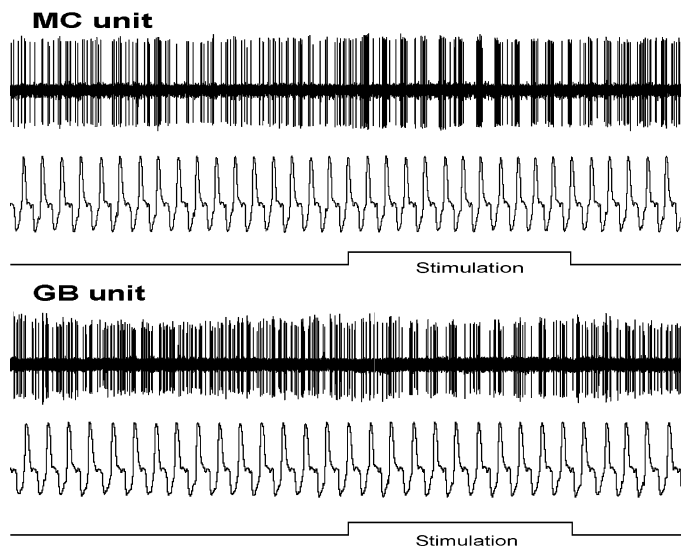
test, 0.05 significance level). Both MCL and EPL units responded most often to ISO and to MAK and least often to FO. The responsiveness of MCL units was also compared with a previous study of mitral cells by Apelbaum and Chaput (2003) where responses to only 6 odors were evaluated; as expected, there were no significant differences in the responsiveness of MCL units in the 2 studies.

When GB and notGB units were compared for responsiveness (Figure 2B), they were found to be significantly different ($P < 0.05$). GB units were more responsive to ISO and MAK (responding 91% of the time) and to CIN, CYM, and HEX.

We next compared the number of odors to which a given unit responded (reactivity) for MCL and EPL units, first using FO, ACE, CIN, ISO, CYM, and MAK, so that the data of Apelbaum and Chaput (2003) on mitral cells obtained with these 6 odors could be included in the comparison (Figure 3), and then using the 8 odors utilized in this study (Figure 4). MCL unit reactivity did not differ significantly (Fisher test, 0.05 significance level) from the study by Apelbaum and Chaput (2003). As shown in Figure 3A, some MCL unit cells did not respond to any of the 6 odors, some responded to only 1, 2, or 3 odors, while the majority of MCL units (69%) responded to 4, 5, or 6 odors. On the other

Figure 1 Example of response of an MCL and a GB unit to the same odorant. Cells were recorded in 2 different rats. The respiratory activity of the rats is shown below the spike activity.
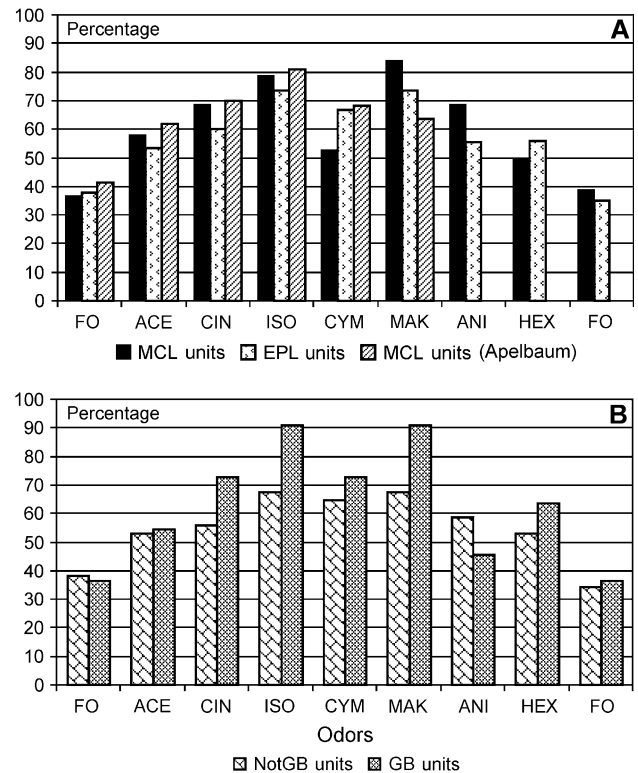

Figure 2 **(A)** Responsiveness of MCL and EPL units. The percentage of MCL and EPL units that responded to each of 9 periods of odor stimulation are plotted along with mitral cell data from Apelbaum and Chaput (2003) for 6 of the odors. **(B)** The percentage of notGB and GB units that responded to each of 9 periods of odor stimulation.

Figure 3 Reactivity of MCL and EPL units to 6 odors. The number of odors that a unit responded to was tabulated. **(A)** The percentage of MCL and EPL units responding to 0, up to all 6 odors is plotted along with data from Apelbaum and Chaput (2003). **(B)** The percentage of notGB and GB units responding to these odors is compared.
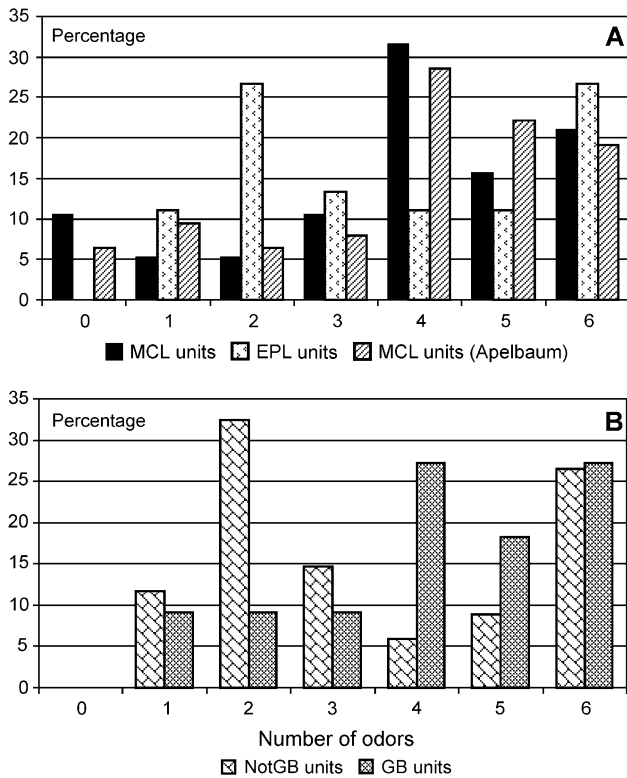

Figure 4 Reactivity of MCL and EPL units to 8 odors. The number of odors that a unit responded to was tabulated. **(A)** The percentage of MCL and EPL units responding to 0, up to all 8 odors is plotted. **(B)** The percentage of notGB and GB units responding to these odors is compared.

hand, all EPL units (GB and notGB combined) responded to at least one odor. Compared with MCL units, a significantly higher proportion (27%) of the EPL units responded to 2 odors and a significantly lower proportion (11%) responded to 4 odors (Fisher test, $P < 0.05$). When GB and notGB units are compared (Figure 3B), the pattern displayed by GB units did not differ significantly (Fisher test, 0.05 significance level) from the pattern of MCL units in that the majority of the units (72%) responded to 4, 5, or 6 odors. NotGB units responded most frequently (58% of the time) to either 2 or 6 odors and GB responded most frequently (73% of the time) to 4–6 odors. Compared with notGB units, GB units responded significantly less often to 2 odors and more often to 4 odors (Fisher test, $P < 0.05$).

MCL units and EPL units were also compared for the 8 odors utilized in this study (Figure 4) because adding 2 new odors to the previous odor set might reduce the proportion of unresponsive cells and/or increase the number of odors inducing a response. As shown by comparing Figures 3A and 4A, the same proportion of MCL units did not respond to any of the 6 odors (11%). When 2 more odors added, MCL units that responded previously to 1 or 2 odors
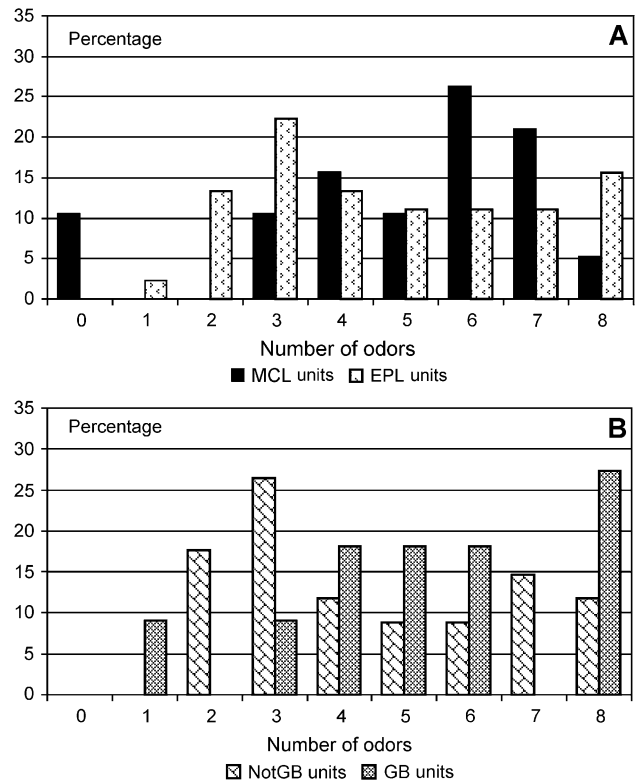
(Figure 3A) responded to both of the new odors, so that now MCL units that responded to odors responded to at least 3. Lastly, the highest percentage (47%) of the MCL units responded to 6 or 7 odors, instead of 4 odors in Figure 3A. As shown by the lack of unresponsive EPL units in Figure 4A, all EPL units responded to at least 1 odor among 8 odorants. Most responded to more than 1 odor, but only 22% responded to 6 or 7 odors (significantly less than MCL units, Fisher test, $P < 0.05$). Thus, odor reactivity was increased more for MCL units than for EPL units by the addition of 2 new odors. When GB and notGB units are compared for 8 odors (Figure 4B), a significantly higher proportion of notGB units (44%) responded to 2 or 3 odors compared with GB units (9%) (Fisher test, $P < 0.05$). On the other hand, 27% of GB units responded to all 8 odors compared with 12% for notGB. Grouped slightly differently, notGB units responded significantly ($P < 0.05$) more often to 1–3 odors than GB units (44% vs. 18%), whereas GB units responded significantly more often to 4 or more odors (82% compared with 66%). GB units were thus more responsive and more responsive to a higher number of odors.

From the responsiveness data to individual odorants (see Materials and methods), we tabulated the number of times that each unit responded to each of the 28 possible pairs of

Figure 5 Responses to odor pairs. The difference in how MCL and EPL units respond to pairs of odors was calculated by subtracting the frequency (percentage) that EPL units responded to an odor pair from the frequency that MCL units responded to that pair. (see text for example).

odorants and then calculated the percentage (frequency) of MCL and EPL units that responded to each odorant pair. These data are shown in Figure 5, where the difference between the response frequencies of MCL and EPL units is plotted for each odor pair. For example, 6 out of the 19 MCL units (32%) responded to both food (FO) and ACE, as did 13 out of 45 EPL units (29%), yielding a difference of 3%. One can see that MCL units responded significantly more often to pairs than EPL units (Fisher test, $P < 0.05$). We also calculated whether some pairs of odors evoked a response more frequently by comparing the number of times a unit that responded to one of the odors responded to a pair of odors (data not shown). No significant differences were observed (Fisher test, 0.05 significance level).

Figure 6 compares the frequency of the CTH patterns during odor stimulation; 311 histograms where odor evoked a response are included. Types 1a and 1b are unsynchronized, 2a and 2b show a simple increase, 3a simple decrease, and 4a–4d complex patterns. Using the Fisher exact test, MCL units are significantly different from EPL, GB, and notGB for patterns 1a and 1b and from EPL and notGB for 2a and 2b. MCL units exhibited patterns 1a, 2b, and 3 significantly more often, patterns 1b and 2a significantly less often, but did not exhibit a 4a–4d response either more or less often (Fisher test, 0.05 significance level).

The 311 CTHs were also used to calculate the mean rate of unit firing per respiratory cycle as well as the maximum firing and the range (difference between the maximum and minimum) per respiratory cycle. These results (Table 1) characterize the overall level of firing of the units during odor responses in reference to the respiratory cycle. Because the distribution of the variables did not follow a normal law, the Wilcoxon, a nonparametric statistical test, was applied. A significant difference at the 0.05% level was found between MCL and EPL units and among MCL, notGB units, and GB units for the 3 parameters. EPL units were significantly more

Figure 6 Comparison of CTH pattern types during odor responses. The percentage of the different cell types exhibiting each pattern type is plotted. Complex patterns 4a–4d were combined. The inset shows the types of CTHs (see text).

active than MCL units, notGB and GB units were both significantly more active than MCL units, and GB units were significantly more active than notGB; GB units had the highest mean and max firing frequencies and the highest range. When considering separately the responses to the different odors, GB units were significantly more active than MCL units for FO, ACE, CIN, ISO, and CYM. Thus, in general, GB units had the highest firing discharges during odor-evoked responses.

Discriminant analysis of MCL and EPL units

To examine in more detail potential responsiveness differences between EPL and MCL units, factorial discriminant analyses were performed on response CTHs. A first discriminant analysis was done to determine whether there was a global difference between MCL and EPL unit populations; these results allowed us to try to determine further whether there was a difference among MCL, notGB, and GB units. The goal was to find the bins that contributed the most to a good discrimination between units.

The discriminant analyses were performed on the 311 responses (CTHs) evoked by odors with the spike activity during each CTH divided into 45 equal bins. The first analysis aimed to separate the MCL and EPL cell types. There was thus 1 classification variable (cell type, a qualitative variable) and 45 quantitative variables (the firing frequencies in the 45 bins). The analysis was used to find 1) a set of linear combinations of the quantitative variables that best revealed differences among the classes of units and 2) a subset of the quantitative variables that best revealed the differences among the cell types. Because the qualitative variable had only 2 modalities (MCL or EPL), the analysis gave only one discriminant variable, called Can1.

After an examination of the scores of Can1 with all odors, the variables (bins) corresponding to small scores in absolute values were removed until the error rate of classification by cross-validation was as small as possible (Lachenbruch and Mickey 1968; Lachenbruch 1979; Dillion and Goldstein 1984; Hand 1986). In this analysis, an error rate of 0.2379, that is, 24% of misclassified units, was reached when 28 variables (bins) were retained. These bins were subsequently utilized in discriminant analyses performed for each odor.

Figure 7A shows an example of an analysis obtained with the 33 responses induced by ACE. For this odor, MCL and EPL units were clearly opposed along the single canonical axis Can1, with all MCL units situated on the negative side of the axis and a majority of EPL units situated on the positive side of this axis. Figure 7B shows how the activity in the 28 selected bins is distributed along the discriminant axis Can1. The x axis shows the coefficients of correlation between the variables (bins) and the solution of Can1 for the responses to ACE; only the 28 selected bins are shown though the correlation coefficients for all 45 bins were calculated. As visible in the figure, bins 37, 13, 17, 4, 14, 6, 1, 11, and 12 were the most positively correlated with Can1. From the position of EPL units on Can1 and the correlation

Table 1 Response mean, max, and range. Values are means ± SEs

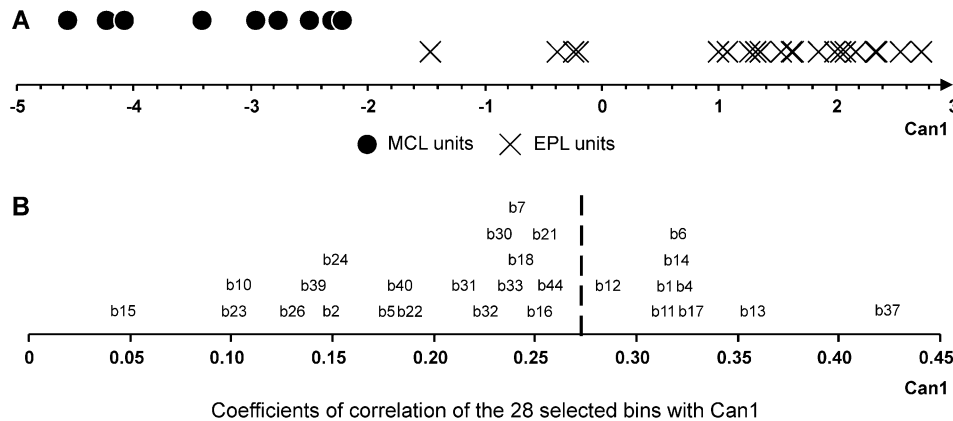| Variable	Cell types			
	MCL	EPL	notGB	GB
Number of responses	97	214	152	62
Mean number of spikes per bin per respiratory cycle	0.2192 ± 0.1572	0.310 ± 0.206	0.2833 ± 0.2087	0.3755 ± 0.185
Max number of spikes per bin per respiratory cycle	0.6977 ± 0.40	0.9127 ± 0.562	0.8675 ± 0.5588	1.0234 ± 0.5588
Range (Max–minimum) number of spikes per bin per respiratory cycle	0.6887 ± 0.3964	0.9006 ± 0.5536	0.8574 ± 0.5499	1.0064 ± 0.5526

Figure 7 Discriminant analysis with one qualitative variable (cell type) and 2 modalities: distribution of MCL (filled circles) versus EPL (*X*'s) units (in **A**) and of the coefficients of correlation of the 28 discriminant bins, b$_i$'s, of the respiratory cycle (in **B**) along the first canonical axis, Can1. In both graphs, symbols or labels were displaced vertically to avoid overlapping. In (B), all variables situated to the right of the dashed line have significant correlation coefficients with Can1.

coefficients of the 45 bins on Can1, we can conclude that the majority of the EPL units have a higher firing activity than the MCL units during the beginning and the second half of inspiration and at the end of expiration.

A similar analysis was done for each of the 7 odors, FO, CIN, ISO, CYM, MAK, ANI, and HEX. The positions of the most correlated bins in terms of the respiratory cycle are presented in Table 2. The EPL units are more active than the MCL units during the middle of inspiration for a majority of odors. They are also more active at the end of expiration for several odors. Thus, EPL units are more synchronized on the middle of inspiration and on the end of expiration. MCL units are more active only with one odor (HEX) during the beginning and end of expiration. It is also clear that the synchronization of the cell activity on the respiratory cycle varies with the odor.

Discriminant analysis on MCL, GB, and notGB units

Because MCL and EPL were clearly discriminated by the previous analysis, a series of discriminant analyses was performed to determine whether the 2 subgroups of EPL units differed in their synchronization with the respiratory cycle. Figure 8 shows the results obtained for ACE. As visible on Figure 8A, all the GB units have positive high values on Can1, whereas all MCL and notGB units have negative values. The 3 groups of cells are thus well discriminated. Figure 8B represents the correlations of the selected variables (bins) with Can1 and Can2. The bins most positively correlated with Can1 were mainly situated in the second half of inspiration (bins 11, 12, 13, 14, 17, 18, and 21) and in the first half of expiration (bins 23, 24, 26, 27, 28, and 28). GB cells were thus more active than MCL and notGB units at the transition between inspiration and expiration. On Can2, the most well-correlated bins were mainly situated at the end of expiration (bins 37, 41, and 42). On this axis, MCL and notGB units were well separated (Figure 8A). As shown by comparing the graphs 8A and 8B, it can be inferred that notGB units were more active than the MCL units during the end of expiration.

Table 3 presents the data for the 8 odors used in this study. GB units were more active than the 2 other cell types during the majority of the different periods of the respiratory cycle.

In the other cases, the notGB cells were more active. In this analysis, MCL units were never more active than both GB and notGB units. The synchronization of the cell activity on the respiratory cycle depends on the odor. For example, with CIN, the GB units were more active than the other cell groups at the beginning and end of inspiration and at the beginning of expiration.

Discussion

Although anatomical differences between mitral and tufted cells of the MOB such as their morphology and central projections are fairly well characterized (e.g., Haberly and Price 1977; Macrides and Schneider 1982; Mori et al. 1983; Orona et al. 1984), relatively little is known of their physiological differences and in particular differences in their responses to odor stimulation. Differences in the spontaneous activity and impulse conduction of mitral and tufted cells were indeed found in the companion paper (Griff et al. 2008), not between mitral and tufted cell populations, but when tufted cells were separated into cells recorded in the core of the EPL (notGB cells) and at the GB (GB cells). The latter had higher spontaneous activity and faster conduction velocities. In the present paper, there were no significant differences between the responsiveness of mitral and all identified tufted cells to the 7 pure chemical odors and rat chow, as measured by the percentage of units that responded to individual odorants (see Figure 2), nor in their reactivity, that is, the number of odors to which they responded (see Figures 3A and 4A). Although there were obvious and potentially interesting differences in these latter graphs, a low number of units in some reactivity groups precluded a complete statistical comparison. Significant differences were mostly between GB and notGB classes (see Discussion below).

Differences were also found when more subtle aspects of the responses were explored, such as the firing rate per respiratory cycle or the distribution of firing activity along the respiratory cycle. EPL units were significantly more active than MCL units. They had significantly higher mean and maximum activity, larger ranges (Table 1), and a higher firing activity during the beginning and the second half of inspiration and at the end of expiration (Figure 7). More clear differences were observed when the subtypes of EPL units

Table 2   Summary of the results of the discriminant analyses done on the responses to each odor

	Inspiration			Expiration		
Activity	Beginning	Middle	End	Beginning	Middle	End
MCL > EPL units				HEX		HEX
EPL > MCL units	ACE, ISO, and CYM	ACE, FO, HEX, MAK, ANI, and CYM	ACE, CIN, and ISO	CIN and CYM	ACE and FO	ACE, MAK, ANI, and CYM

It indicates which cell group (MCL or EPL unit) was significantly the more active during each period of the respiratory cycle and for which odor. As visible, EPL units were more active than MCL units during all periods of the respiratory cycle for all odors, except HEX.

were examined separately. Both GB and notGB units had significantly higher activity than mitral cells, and GB units had the highest activity.

The higher activity of EPL units is in agreement with Nagayama et al. (2004). However, their study differed from

Figure 8 **(A)** Discriminant analysis with 1 qualitative variable (cell type) and 3 modalities: MCL (filled circles) versus GB (filled triangles) versus notGB (open triangles) units: plot of first 2 discriminant axes, Can1 and Can2. **(B)** Distribution of bin activity along the 2 discriminant axes: the coefficients of correlation of the 28 discriminant bins are plotted on Can1 and Can2. Bins to the right of the dashed line are significant along the Can1 axis; bins 37, 41, and 42 are significant along the Can2 axis.

the present in several aspects: 1) they compared odor responses of mitral cells with the responses of middle tufted cells only; 2) their recordings were restricted to the aliphatic acid-aldehyde–responsive area in the dorsomedial part of the MOB, whereas our recordings were performed in the whole MOB; 3) they excluded superficial tufted cells such as GB cells of the present study; 4) they utilized only homologous series of aliphatic acids and aldehydes as odorants; 5) they did not analyze the responses with respect to respiration although it has been shown extensively that the odor-induced activity of MOB units is synchronized on respiration (see review in Buonviso et al. 2006; Scott 2006) and cannot be analyzed correctly without taking into account this parameter (Chaput and Holley 1980; Chaput 1986). Nonetheless, they also found that middle tufted cells had higher firing rates than mitral cells. As proposed by Nagayama et al. (2004), ''mitral and tufted cells may use different firing rates for transmitting the olfactory signals to their target neurons.''

Further comparisons with the literature are difficult because only a few studies have been performed on the odor responsiveness of identified mitral and tufted cells in mammals and/or in similar experimental conditions. Doving (1987) showed little difference between the distribution of responses into excitation, inhibition, or no response of the odor-evoked activity of mitral and tufted cells identified by their antidromic latencies to LOT stimulation. His aim was to determine how the mammalian olfactory system responds to a variation in odor concentrations and its reaction to prolonged odor stimulation. Thus, his study was performed in tracheotomized rats stimulated by tracheal depression using long stimulation periods with slowly increasing concentration of odor and a monitored nasal air flow. No differences were observed between the responsiveness of mitral cells in this study and in a previous study by Apelbaum and Chaput (2003). Visual inspections of the reactivity to the 6 odors used in this latter study did not reveal any obvious difference in the patterns from the 2 sets of mitral cell data. In both studies, some units did not respond to any odor, and this did not change when 8 odors were considered. On the other hand, all tufted cells responded to at least one odor. When responses to odor pairs were examined (Figure 5),
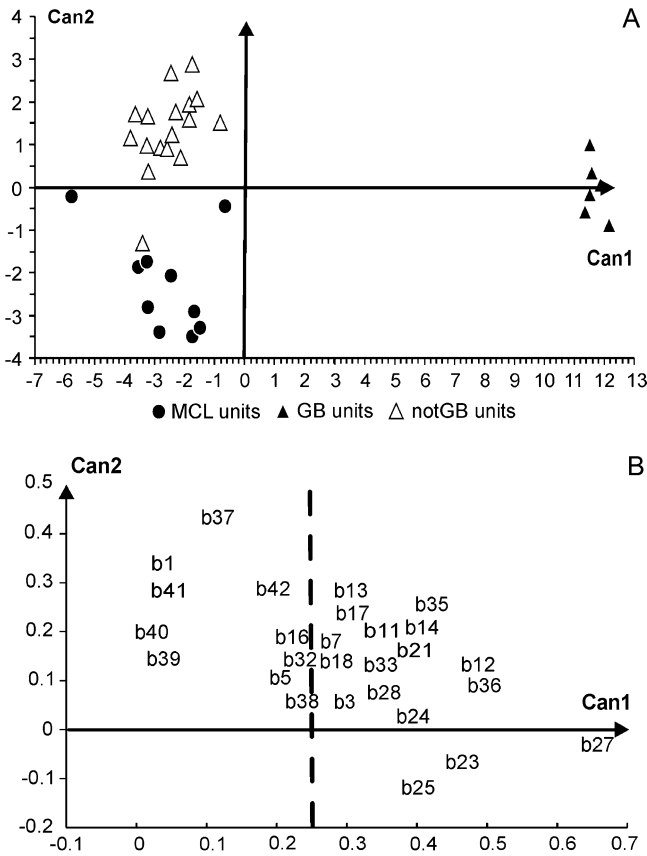
Table 3   Summary of the results of the discriminant analyses done on the responses to each odor

	Inspiration			Expiration		
Activity	Beginning	Middle	End	Beginning	Middle	End
GB > notGB > MCL	CIN		CIN and MAK	CIN		MAK
GB > notGB and MCL		ACE and F0	ACE	ACE	ACE and FO	
GB > MCL > notGB	HEX and ANI		ISO			HEX, ISO and ANI
notGB > GB and MCL		CYM	CYM	CYM		CYM
notGB > MCL > GB		ISO	HEX			

It indicates which cell group (MCL, notGB, or GB unit) was significantly more active during each period of the respiratory cycle and for which odor.

mitral cells responded significantly more often. These data could suggest that tufted cells might be more selective and mitral cells might be more involved in comparing different odors.

In the present study, each response was associated with a particular CTH pattern and the frequencies of such patterns were compared (Figure 6). Although the frequencies of some patterns differed between MCL and EPL units, other patterns did not, and it is difficult to formulate a general conclusion. A more detailed analysis of the CTHs involved bin-by-bin discriminant analyses (Figures 7 and 8). The goal of this analysis was to discriminate between EPL and MCL units, and the analysis clearly separated these units, as well as the GB and notGB units. When all odors were considered, most EPL units were positive on the discriminant axis Can1, indicating that the EPL units are more active than the MCL units. This result is in agreement with our analysis of the mean, max, and range of firing. When the discriminant analysis was applied to the responses to each odor, the synchronization of the cell's activity on the respiratory cycle depended on the specific odor. However, for a majority of odors, EPL units were more active than the MCL units during the middle of inspiration (Table 2). Some were also more active at the end of expiration for several odors. Thus, the EPL units are more synchronized on the middle of inspiration and on the end of expiration. MCL units are more active only with one odor (HEX) during the beginning and end of expiration. It is not clear whether this pattern of response by mitral cells is related to the chemical properties of this odor.

For 11 tufted cells, electrode track reconstructions allowed identification of cells at the EPL-GB. GB cells were significantly more responsive to 2 odors (Figure 2B): ISO and MAK. Both are linear ketones and might be less discriminated, as reported in a comparative study of rat and frog responsiveness of olfactory neuroreceptors (Duchamp-Viret et al. 2000). The higher responsiveness to ISO and MAK is likely not due to the ketone function because ACE, which is also a ketone, but with an aromatic ring, did not induce a higher number of responses than the nonketonic odorants. Another suggestion that GB units may be less involved in odor discrimination is their increased reactivity from 6 to 8 odors. Indeed, the same percentage of GB and notGB cells responded to all 6 odors, whereas more notGB units responded to only 2 odors. On the other hand, with 8 odors considered, a significantly higher percentage of GB units responded to all 8 odors (Fisher test, $P < 0.05$), whereas a higher percentage of notGB units still responded to only 2 or 3 of the 8 odors.

As shown by the overall levels of firing of the cells reported in Table 1 and the distribution of their firing activity along the respiratory cycle (Figure 8), GB units had higher activity than notGB and MCL units during odor presentation. GB units showed greater activity at the transition between inspiration and expiration, whereas notGB units were more active at the end of expiration. The activity pattern of GB units to odors is in agreement with results on spontaneous activity presented in the companion paper (Griff et al. 2008). GB units were found to have a higher mean spontaneous activity than MCL units, and discrimination analysis showed that their higher activity was during inspiration. Thus, superficial projection tufted cells, and possibly other external tufted cells (Wachowiak and Shipley 2006), are more active and more synchronized on inspiration. Higher odor-induced firing rates were already observed by Nagayama et al. (2004) in middle tufted cells. Both the higher firing activity and the stronger synchronization on respiration reported in our study likely can be ascribed to differences of connectivity. Mitral cells have long secondary dendrites and their extensive dendrodendritic connections with granule cells in both the EPL and the deeper layers of the MOB are in good agreement with more intense control of their activity and reactivity by the inhibitory intrabulbar circuits. In contrast, middle tufted cells have shorter secondary dendrites and external tufted cells, adjacent to the glomerular layer, have relatively short, asymmetric dendritic fields (Mori et al. 1983; Orona et al. 1984). Due to this functional sublaminar organization of the EPL, the closer the glomerular layer the somata of MOB output cells are the less intense the inhibitory control by intrabulbar circuits.

The picture that emerges is that GB units, projection-type tufted cells recorded near the GB, may form a distinct physiological class of output neurons. Tufted cells in general have a lower threshold to olfactory nerve electrical stimulation (Schneider and Scott 1983). Thus, GB units may provide lower threshold responses to odor stimulation that reaches central structures more quickly due to the higher impulse conduction velocity. This pathway could be used for the initial detection of odors and potentially could trigger the sniffing response for odors deemed relevant by cortical or subcortical structures. The relative lack of inhibition in tufted cells by other odors in a mixture (Nagayama et al. 2004) may allow more initial odor signals to reach the cortex where subsequent analysis via a mitral cell input could refine the information.

Thus, the overall conclusion of the present studies is that mitral and tufted cells in general are similar in their impulse conduction velocity, their spontaneous activity, and in their responses to odors but that differences appear when the specific location of their somata in MOB layers are taken into account. Indeed, identified tufted cells located at the EPL-GB have a significantly faster impulse conduction velocity and higher spontaneous activity, particularly during inspiration, and are more responsive to odorants. Further studies using, for example, different odor concentrations and/or mixtures are needed to further understand the respective roles of the subclasses of MOB output cells.

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