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Olfactory Bulb Output Cell Temporal Response Patterns to Increasing Odor Concentrations in Freely Breathing Rats

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Abstract

This study compares the single-unit responses of 74 mitral/tufted cells recorded in freely breathing rats to step increases of the intensity of five odorants from 2×10^{-4} to 10^{-1} of saturated vapor pressure. It reveals a stability of the responses of these olfactory bulb output cells. Olfactory stimulation has frequently been shown to produce a strong patterning of mitral/tufted cell discharges highly correlated with respiration. In this study, cells were generally found to show the same response type to two consecutive concentrations, and only a few cells switched their response from excitation to suppression or vice versa. Their firing peak and/or trough occupied the same position in a high proportion of respiratory cycles recorded during a stimulation, and they remained significantly time-locked to the same respiratory epoch for the next higher concentration. Increasing odor concentration did not cause the mean firing frequency of individual cells during a peak to change appreciably between successive or extreme concentrations. By contrast, it tended to shift their maximum frequency during this peak towards an earlier respiratory cycle after stimulation onset. These results are compared with data reported in other electrophysiological studies and with results given by olfactory bulb models before being discussed for their implications in odor coding.

Introduction

In olfaction, one important hypothesis supposes that odorant quality information is encoded in the olfactory bulb (OB), the first relay of the olfactory pathways, by the spatio-temporal distribution of the mitral/tufted (M/T) cells co-activated by an odor. Considerable evidence that different odorants produce different patterns of neural activity in the OB has been accumulated in the past by analyzing M/T cell degeneration following long-duration odor exposures (Doving and Pinching, 1973; Pinching and Doving, 1973; Laing and Panhuber, 1978), or by using 2-deoxyglucose (Sharp *et al.*, 1975, 1977; Stewart *et al.*, 1979; Jourdan *et al.*, 1980) or voltage-sensitive dyes (reviewed in Kauer, 1991). More recently regional differences in OB responsiveness for aliphatic and aromatic compounds have been reported using M/T cell single-unit recordings (Imamura *et al.*, 1992; Katoh *et al.*, 1993), and functional mappings using c-fos expression (Guthrie and Gall, 1995) have both confirmed the specificity of the patterns evoked by different odorants and revealed that these patterns remain globally similar when manipulating odor concentrations, even if a broadening of the labeled zones occurs when increasing stimulus intensity.

In theory, this spatial pattern appears to be incompatible with individual M/T cell response changes with odor intensity observed in almost all studies performed in fish (Meredith and Moulton, 1978; Meredith, 1981), amphibians (Doving, 1964; Higashino *et al.*, 1969; Kauer, 1974; Kauer and Shepherd, 1977; Duchamp, 1982; Duchamp and Sicard, 1984; Hamilton and Kauer, 1985) or mammals (Moulton, 1963; Mathews, 1972; Mair, 1982a; Harrison and Scott, 1986; Meredith, 1986; Reinken and Schmidt, 1986; Chaput and Lankheet, 1987), except for the fact that the divergent results reported in these studies require us to reconsider the issue of changes in M/T responses. Thus we decided to revisit M/T cell response changes with odor intensity. This was done in freely breathing rats rather than animals placed under an artificial sniff paradigm, as was done in most of the electrophysiological studies reported above, since respiration, which plays a crucial role in the expression of odor-evoked bulbar patterns (Chaput and Holley, 1980; Chaput, 1986; Sobel and Tank, 1993), was assumed to be an important factor for the stability of M/T cell responses. However, animals were anesthetized, as for most of the above-mentioned studies performed in mammals, so as to reduce the changes in respiratory cycle duration which may have blurred the effects of odor intensity changes. In these experimental conditions, OB responsiveness was found to increase with odor intensity, and individual M/T cell responses showed relatively limited changes.

Materials and methods

Details of the different procedures presented below have been described previously (Chaput and Holley, 1980; Buonviso and Chaput, 1990; Buonviso *et al.*, 1992; Chaput *et al.*, 1992) and will only be given in brief here.

Surgical methods

Thirty-five adult Wistar rats (250–300 g) were used in this study. Animals were anesthetized by i.p. injection of Equithesine (a mixture of pentobarbital sodium and chloral hydrate) with an initial dose of 3 ml/kg. Anesthetic was supplemented as necessary to maintain a deep level of anesthesia, as determined by the lack of withdraw reflex of the leg in response to a moderately intense toe pinch. Rectal temperature was monitored and maintained at 37 ± 0.5°C by a regulated heating pad and surgical wounds of the animals were regularly infiltrated with 2% Procaine.

Recording procedures

The respiratory activity was recorded through a thermistor. The signal was fed to an amplifier and filter (0.1–30 Hz band-pass), and stored on a digital tape recorder (DTR, Biologic Scientific Instruments, Claix, France).

Single-unit action potentials were recorded using glass micropipettes (8–30 MΩ) filled with 2% pontamine sky blue (Aldrich) in 2 M NaCl. The recorded signal was led through a conventional amplifier, filtered between 300 and 3000 Hz, and stored on the DTR along with signals indicating odor delivery.

Odor stimuli

Five reagent-grade chemicals were utilized as stimuli: camphor, cineole, isoamyl acetate, limonene and methyl-amyl ketone.

Odors were delivered with a flow dilution olfactometer described in detail elsewhere (Vigouroux and Chaput, 1988). Briefly, the nozzle of the olfactometer was continuously supplied with a main flow of pure and humidified air (28 l/min). A second flow of pure air (2 l/min) was injected into this flow between odor deliveries and it was replaced by an equivalent flow of odorized air during stimulation. This last flow was initiated 10–15 s before odor delivery so as to allow odor concentration to stabilize in the line, and it was exhausted until stimulation onset.

Experimental protocol

Electrodes were lowered in the ventral mitral cell layer, as determined by the appearance of a dipole reversal in the field potentials evoked by stimulating the M/T cell axons in the lateral olfactory tract and by the occurrence of large-amplitude spikes (Phillips *et al.*, 1961, 1963). It was confirmed by depositing the dye contained in the micropipette at the recording site using a negative current of 2–5 μA passed for 10 min (10 s on, 10 s off), and by verifying histologically the position of the dye spot on 40-μm-thick serial frozen sections of the bulb stained with cresyl violet. Only cells recorded in the mitral cell layer were retained in this study.

Once a single unit was isolated, its responsiveness to the five odorants delivered at the medium concentration of 2×10^{-2} of saturated vapor pressure was first determined. Then odorants that had evoked a response were applied in ascending order of concentration. Concentrations tested were 2×10^{-4}, 6×10^{-4}, 2×10^{-3}, 6×10^{-3}, 2×10^{-2}, 6×10^{-2} and 10^{-1} of saturated vapor pressure. Both the control-presentations of the five odorants (abbreviated as C in the text) and the test-presentations of odor concentrations (numbered 1–7 in the text) lasted 10 s and were separated by intervals of at least 2 min. They were initiated 10 ms after the transition between inspiration and expiration, so that the first respiratory cycle included in the stimulation would correspond to a complete stimulation period. When several odorants evoked a response, all concentrations of a single odorant were presented before proceeding to the next one.

Data analysis

During experiments, signals were systematically stored for subsequent analysis on a computer connected to a CED-1401 Plus data acquisition system (Cambridge Electronic Design Ltd, UK). Cell activity was digitized at 15 kHz to later extract spikes using the Spike2-CED software for data acquisition and analysis. Respiration was sampled at 1000 Hz and stimulation events were stored as their time of occurrence with respect to the beginning of acquisition.

As exemplified in Figure 1, olfactory stimulation has been extensively shown to produce a strong patterning of OB single-unit spikes highly correlated with respiration (Adrian, 1950; Chaput and Holley, 1980, 1985; Chaput *et al.*, 1992; Sobel and Tank, 1993). Thus all analyses of spike activity were performed with respect to the respiratory cycle. Most of them have been already described in our previous publications (Chaput and Holley, 1980; Buonviso *et al.*, 1992; Chaput *et al.*, 1992) and will only be briefly summarized here.

The respiratory signal was first processed to discriminate the positions of the beginnings and maximum of inspiratory phases, the transitions between inspiratory and expiratory phases, the maximum of expiratory phases, and the return of the signal to its base level. Then, the spike activity of each cell before, during and after each stimulation was represented on the computer screen with the use of a raster plot and firing frequency histograms triggered on inspiration beginnings (Figure 1). This activity was processed as follows to detect the existence of a response and to determine its type without reference to the other responses in the series and without knowledge of the stimulus concentration. In a first step, the crude list of interspike intervals of the cell was analyzed by computer to locate

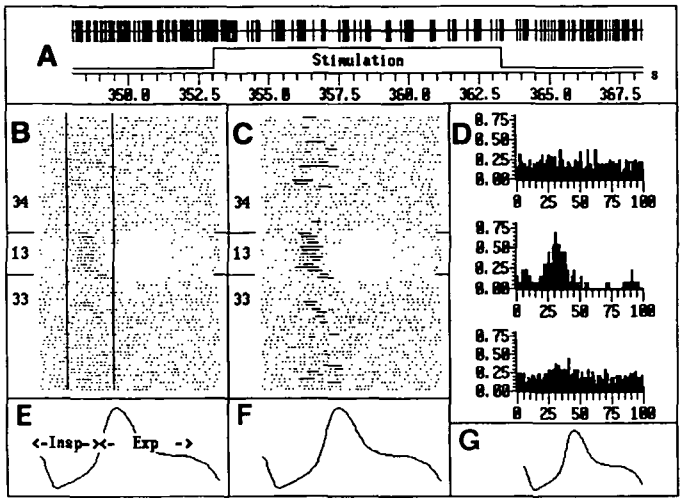

Figure 1 This figure illustrates the principal steps utilized to determine the existence of a firing peak in the response of a cell to a stimulation (here with ISO at 2 × 10^{-2} of saturated vapor pressure). In the window **(A)** of the computer screen is first shown the spike activity of the cell before, during and after the stimulation. This activity is represented respectively in **(B)** and **(D)** as a raster plot and as CTHs triggered on inspiration beginnings, whereas the averaged form of the corresponding respiratory cycles recorded during the same period is presented in **(E)**, **(F)** and **(G)** (Insp: inspiratory phase, Exp: expiratory phase). Then, spikes are grouped into bursts, their positions in the successive respiratory cycles recorded before, during (between the two horizontal lines in B and C) and after stimulation being represented with respect to inspiration beginnings. Two vertical cursors are then positioned in B to define the approximate boundaries of the zone where a firing increase is suspected to occur and the bursts of spikes situated between these limits are selected using the Spike2 facilities and visualized as short horizontal lines in **(C)**. The top, middle and bottom cycle-triggered histograms in D correspond to the periods before, during and after stimulation respectively.

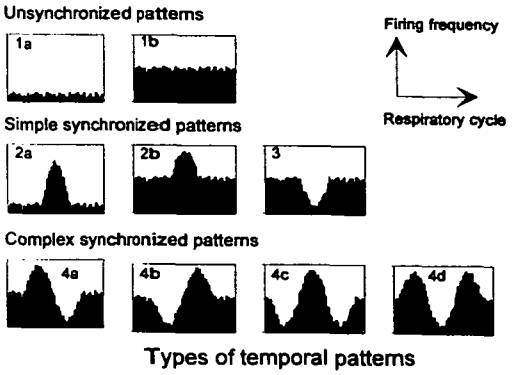

Figure 2 Schematic representation of the most-often observed mitral cell response patterns. They are represented as firing frequency histograms triggered on the respiratory cycle.

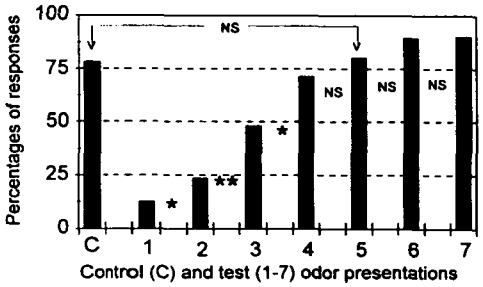

Figure 3 Percentages of responses to control- (C) and test-odor presentations of increasing intensities (1–7). Significant differences between pairs of consecutive concentrations, as determined using the χ^2 test are noted by * ($0.05 > P > 0.01$) and ** ($0.01 > P > 0.001$).

firing peaks or troughs using the facilities offered by the CED software, and to select those that were time-locked to periods of the respiratory cycle where the cell was expected to respond, as determined using the appearance of its raster plot and the form of its cycle-triggered histograms (CTH). The list of these events was stored to further analyze the positions of peaks and troughs within individual respiratory cycles. Then a response was said to occur when the form of the CTHs differed significantly between the pre-stimulation and stimulation periods (Kolmogorov–Smirnov two-sample test, $P < 0.05$), and/or when the proportion of peaks or troughs changed significantly during these periods (χ^2 test, $P < 0.05$). Lastly, the odor-evoked CTHs of responsive cells were assigned to one of the different pattern types already utilized by ourselves in previous studies (Buonviso *et al.*, 1992; Chaput *et al.*, 1992). As shown in Figure 2, they were categorized as unsynchronized, simple synchronized or complex synchronized patterns whether they corresponded respectively to a uniform distribution of activity along the respiratory cycle, to a single change in activity towards excitation (types 2a and 2b) or suppression (type 3), or to a more complex succession of excitations and suppressions.

Results

Data were obtained from a total of 74 neurons that responded to the control presentation of at least one of the five odorants. Fifty-one cells submitted to the whole range of odor concentrations were basically included in this study, and 23 cells that were lost before being submitted to the complete concentration protocol were utilized when possible.

Figure 3 shows the percentage of responses to control- (C) and test- (1–7) odorant presentations. As visible by comparing bars labeled C and 5, cell responsiveness did not differ significantly (χ^2 test, $P < 0.05$) whether the same concentration of an odorant was presented among different odorants or in a series of increasing concentrations. This responsiveness increased significantly with odor intensity between 2 × 10^{-4} and 6 × 10^{-3} (bars 1–4), and then continued to increase (though not significantly) until 6 × 10^{-2} of saturated vapor pressure (bar 6). However, none of the two

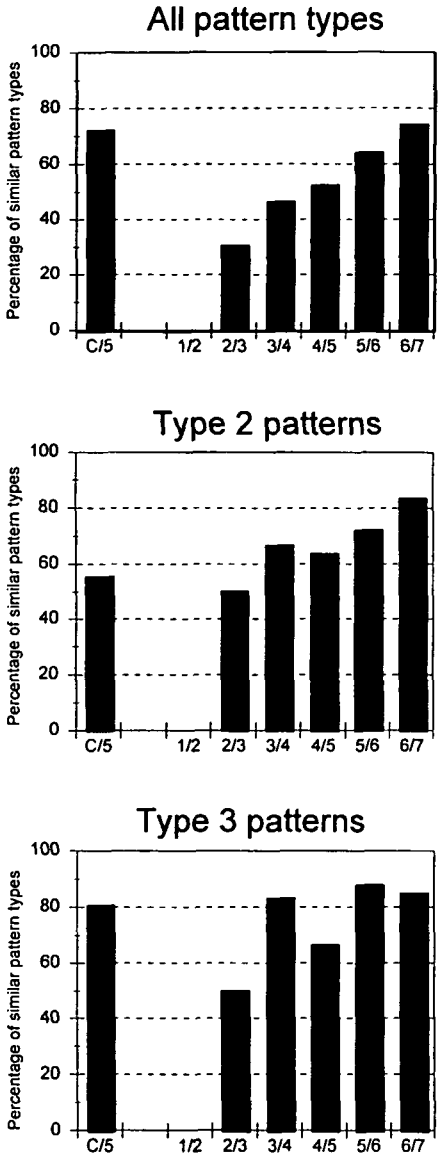

Figure 4 Percentages of similar response patterns when considering all types, or more specifically type 2 or type 3 patterns. The first bar (C/5) of each histogram gives the percentage of cells that showed the same type of response to the control- and test-presentation of the same stimulus at 2×10^{-2} of saturated vapor pressure. The other bars correspond to pairwise comparisons between two successive concentrations. No significant difference was observed between consecutive percentages, as determined using the χ^2 square test ($P < 0.05$).

highest concentrations (bars 6 and 7) were able to recruit all cells.

Figure 4 reveals an overall stability of cell response types. They did not change in 70–80% of cases between control- and test-presentations of the same stimulus, as shown by bars labeled C/5. The proportion of similar types in responses to 6×10^{-4} and 2×10^{-3} of saturated vapor (bars labeled 2/3) was relatively low when considering all types

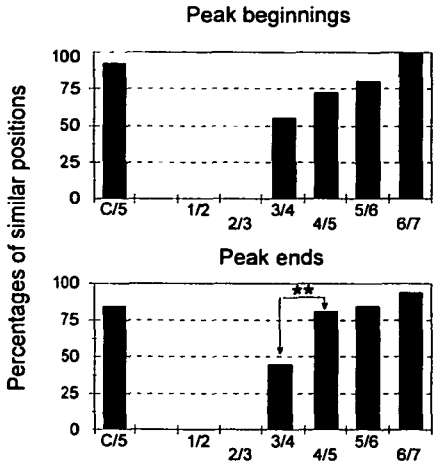

Figure 5 Similarity of peak beginnings and ends in responses to control- and test-presentations of the same odorant at 2×10^{-2} of saturated vapor pressure (bars C/5) and in pairs of test-presentations of increasing intensities (1/2 to 6/7). Significant differences between percentages, as determined using the χ^2 test, are noted by * ($0.05 > P > 0.01$) and ** ($0.01 > P > 0.001$).

(upper diagram) and increased progressively with odor intensity, but without becoming completely identical for the two highest concentrations (bars 6/7). Similarity was immediately higher when considering only the simple synchronized excitatory (type 2) or suppressive (type 3) pattern types, as shown by bars labeled 2/3 in the middle and bottom diagrams. In both cases, the similarity increased for the next higher concentration (bars 3/4) and then did not change significantly for higher pairs of concentrations (bars 5/6 and 6/7).

Since the classification into the types utilized above did not permit us to assess whether the positions, durations and other characteristics of peaks and troughs remained stable or changed with odor intensity, a detailed analysis of these events was performed separately. For this, peak and trough beginnings and ends were attributed to one of the six periods used to subdivide the respiratory cycles.

As shown by bars labeled C/5 in Figure 5, >80% of firing peaks evoked by control- and test-presentations began and finished at the same moment of the respiratory cycle (bars C/5). Too few peaks were induced by concentrations $<2 \times 10^{-3}$ to be included in this analysis. By contrast, ~50% of peaks contained in responses to intermediate concentrations (2×10^{-3} and 6×10^{-3}) had similar beginnings and ~40% of them presented similar ends. This similarity increased for higher concentrations (bars 4/5 to 6/7) and reached 100% for peak beginnings at the two highest concentrations (bars 6/7).

This increased similarity was not accompanied by an increase in the proportion of cycles containing a peak (not shown), nor by a substantial increase of the firing frequency reached in the peak (Figure 6). Whenever a cell was found to show a firing peak during a given epoch of the respiratory

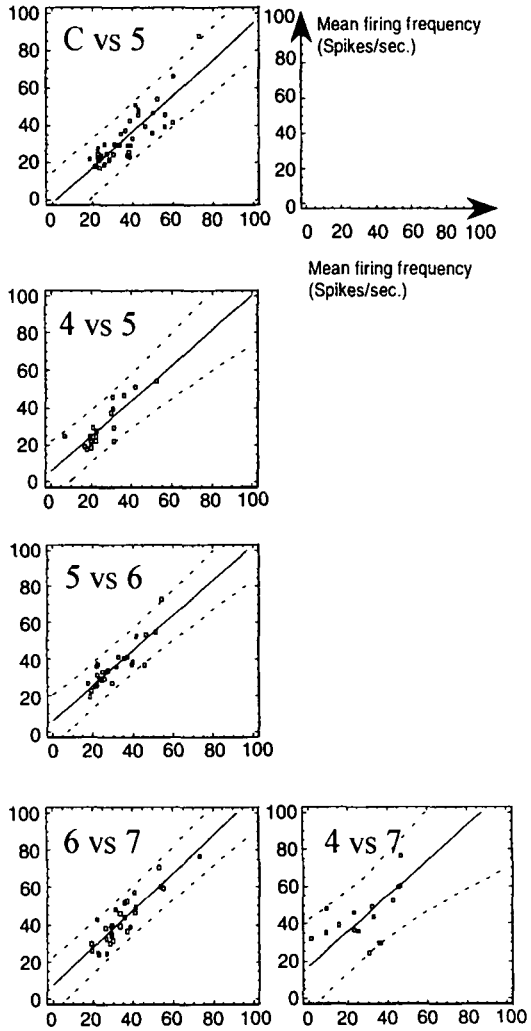

Figure 6 *X–Y* plots of the mean firing frequencies of cells that responded with a peak of activity. The regression curves and the 95% confidence limit curves are represented by full and dashed lines respectively. The upper left plot presents frequencies obtained from responses to control- and test-stimuli at 2×10^{-2} of saturated vapor pressure. The three other left-hand diagrams present frequencies obtained for successive step concentrations ranging from 6×10^{-3} (4) to 10^{-1} (7) of saturated vapor whereas the right-hand diagram plots frequencies obtained for these two extreme concentrations.

cycle, this peak occurred repeatedly during a majority of the cycles recorded during its response whatever the concentration. Proportions of cycles containing a peak ranged between 80 and 100% for the different odor presentations, and did not differ significantly between concentrations (χ^2, $P < 0.05$).

Figure 6 depicts how the mean firing frequencies of the cells in the peaks evolve. It presents *X–Y* plots of these firing activities in the control- and test-odor presentations (C versus 5), in successive pairs of concentrations (4 versus 5 to 6 versus 7), and between the most extreme concentrations (4 versus 7). Frequencies were not found to differ between

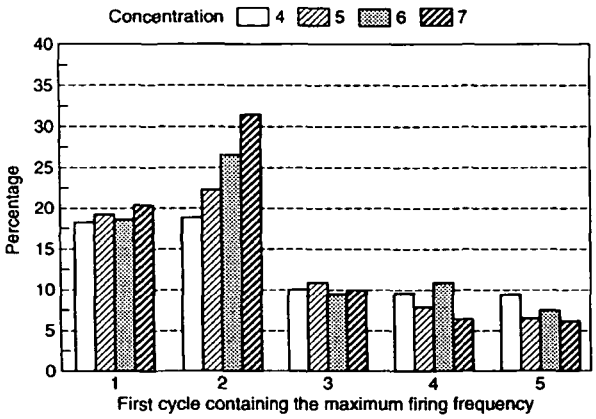

Figure 7 The percentage of cases in which the maximum firing frequency reached by the cells in a peak was situated in one of the five first respiratory cycles following stimulation onset for the four highest concentrations utilized in this study.

control- and test-presentations (C versus 5), nor between successive pairs of concentrations, as shown by the slopes and positions of regression curves and 5% confidence limit curves plotted on the diagrams. This does not mean that no firing change occurred between the most extreme concentrations, as shown in the rightmost lower plot in Figure 6. However, it represented an augmentation of only 13% of the mean firing frequencies of the cells.

In order to determine whether cells responded increasingly earlier after stimulation onset to increasing odorant intensities, we determined in which respiratory cycle the cells reached their maximum frequency for the first time. As shown in Figure 7, this peak of maximum frequency occurred generally in the first or second cycle following the beginning of stimulation. Increasing stimulus concentration tended to shift it from later respiratory cycles to the second one after stimulus onset, but not to the first one.

Troughs were also found to occupy reproducible positions on the respiratory cycle. As can be seen in Figure 8, they began and/or ended at the same moment of the respiratory cycle in ~75% of responses to control- and test-presentations of the same odorant at the same concentration (bars C/5). Responses to 2×10^{-4} and 6×10^{-4} of saturated vapor pressure (bars 1/2) did not contain similar troughs. By contrast, similar troughs were induced by 6×10^{-4} and 2×10^{-3} (bar 3/4), and this similarity was enhanced by odor intensity. It increased progressively for trough beginnings but without reaching 100% as peak beginnings did, whereas it increased immediately to ~65% for trough ends and did not change significantly for further concentrations (χ^2, $P < 0.05$).

Odor concentrations were found not to modify significantly the proportion of respiratory cycles recorded during a stimulation that containing a trough (not shown).

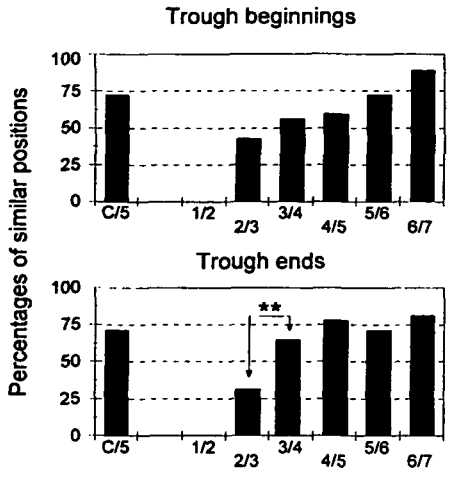

Figure 8 Similarities of trough positions on the respiratory cycle. The conventions used are the same as in Figure 5.

Whenever cells show a trough during a given epoch of the respiratory cycle, this event was present in 80–100% of the respiratory cycles recorded during stimulation, and this proportion did not change significantly with concentration (χ^2, $P < 0.05$).

Lastly, in an attempt to compare our results with the predictions of Meredith's model (1992), we analyzed the frequencies of passage from a peak to a trough or vice versa (not shown). Among cells submitted to a complete stimulation protocol, none was activated by low intensities (2×10^{-4} and/or 6×10^{-4}) and suppressed by high intensities of the same stimulus. Three cells out of 17 (17.6%) were suppressed by low concentrations of an odorant (2×10^{-4} and/or 6×10^{-4}) and activated by higher concentrations; and two cells out of 18 (11.1%) were suppressed at low intensities, activated at intermediate intensities and suppressed again at high intensities.

Discussion

The main findings of this study performed in freely breathing animals are the stability of the respiratory cycle-linked M/T cell response patterns to step changes in odor concentration and the lack of important changes in the firing activity of individual cells. From threshold to the highest concentrations, M/T cells generally present the same response pattern, as shown by (i) the reproducibility of the positions of their firing peaks and troughs with respect to the respiratory cycle; (ii) the consistency of their response types to the highest odor intensities; (iii) their moderate firing increase to increasing odor concentrations; and (iv) the shift of their maximal reactivity towards the beginning of the stimulation. Thus we assume that the main spatio temporal features of the bulbar message evoked by an odorant are likely preserved when its intensity increases.

Comparison with other studies

The M/T cell recruitment observed in this study is in agreement with the results of a majority of earlier studies (Moulton, 1963; Reinken and Schmidt, 1986; Chaput and Lankheet, 1987), and with the model of Anton *et al.* (1991). Generally, the number of responding M/T cells has been shown to increase monotonically with olfactory nerve stimulation frequency in a sigmoidal fashion with an off-range, a near-linear response range and a saturation range. The increase in OB responsiveness described in this study depends on concentration. This recruitment is substantial between 2×10^{-4} and 2×10^{-2} of saturated vapor pressure, where it represents an ~6-fold multiplication of the population of responsive neurons, while the recruitment is smaller for higher concentrations. The form of this concentration–response curve may result from a differential efficiency of odor molecules for stimulating the olfactory receptors. Indeed, there is considerable evidence that the sorption of odorant molecules into the mucosa dictates the extent to which olfactory receptors are activated (Mozell, 1970; Mozell and Jagodowicz, 1973; Hornung *et al.*, 1980; Hornung and Mozell, 1981). To explain this non-linearity of cell recruitment, we suggest that some physical and/or biochemical properties related to the sorption of odor molecules on the olfactory and/or respiratory mucosa might reduce to a greater extent the efficiency of low concentrations.

Regarding the stability of suppressive responses, our results are in agreement with the results of most earlier studies (Mathews, 1972; Kauer, 1974; Kauer and Shepherd, 1977; Daval and Leveteau, 1982; Mair, 1982a,b; Reinken and Schmidt, 1986; Chaput and Lankheet, 1987; Doving, 1987; Hamilton and Kauer, 1989). For example, Kauer observed in the frog that when odor concentration is manipulated for a unit showing a suppressive activity at one concentration, this unit tends to show a qualitatively similar suppression at other concentrations. Likewise, Mathews (1972) has shown in the rat that inhibition varies only in duration and intensity in suppressed cells, depending on stimulus concentration.

Stability of the peaks is in agreement with the examples of M/T cell responses to aliphatic compounds shown by Imamura *et al.* (1992) in a recent paper. By contrast, it disagrees with the intensity effects generally reported in the literature. Kauer (1974) in the frog and more recently Scott and collaborators in the rat (Harrison and Scott, 1986, Wellis *et al.*, 1989) observed more drastic changes. The latter authors observed that M/T cell responses change significantly with concentration, such that their form across the concentration range could not be predicted from their form at any one concentration. This difference with our results may result from a difference of experimental conditions. Their animals were all stimulated using an artificial cycle paradigm whereas our rats were left freely breathing. We

argue that maintaining the natural rhythmicity of the stimulation may modify the dynamic of the nasal airflow less than a tracheal aspiration, so that the variability of the response of the neuroreceptors will be more limited, and the stability of bulbar excitatory responses will accordingly be increased. The results of Hamilton and Kauer on the salamander (1985, 1989) support this hypothesis, since they indicate that changes from excitation to suppression or vice versa are generally not observed with reproducible stimuli.

The stability observed in this study is not consistent with Meredith's data (1986), nor with the predictions of his model (1992). Half of the cells he recorded changed response type at some point over the 2–3 log-unit range tested, but it must be noted that his experimental conditions differed profoundly from ours. For example, he used longer duration stimuli. This might emphasize inhibitory events that we might not see because they are obscured by the excitation occurring with the next respiratory cycle. Furthermore, he used single odor pulses and was analyzing data for a substantial period after the stimulus, which may explain at least partly the difference with our observations. His model predicted passages between excitatory and suppressive responses. On the contrary, in this study no cell was found to pass from excitation to suppression between intermediate and high concentrations, or to show a suppression at low concentrations, an excitation at intermediate concentrations and again a suppression at high concentrations. This may result from differences between our data and those taken into account by Meredith to build his model.

As outlined by Meredith (1986), calculating the magnitude of responses and plotting intensity–response curves are problematic with temporally patterned responses. This led us to calculate the firing frequencies of the cells only on the portion of the respiratory cycle that contained the peak of activity, and to compare firing frequencies exclusively between responses showing a peak of activity at the same moment of the respiratory cycle. Using such precautions, cells were found to have relatively stable firing frequencies over the intensity range utilized in this study, whereas they tended to reach their maximum frequency in an earlier respiratory cycle after stimulation onset. This stability appears to contradict the concentration–response curves obtained by Mathews (1972) in rats and Moulton (1963) in rabbits, and with the examples of M/T cell responses to increasing odor concentrations given by Imamura and co-workers (1992) in their paper on aliphatic compounds. In contrast, it corroborates the observations done by Mair (1982a) and Doving (1987). For instance, the latter author reported that M/T cell discharge increases abruptly at the beginning of the ramp stimulation period, and then remains stable during the rest of the stimulation in 44% of odor presentations.

The trend of the maximum frequency reached by the cells during stimulation to shift towards earlier respiratory cycles and the stability of temporal patterns from cycle to cycle have not yet been reported. This suggests that when intensity increases, the main features of the spatial pattern of activity evoked by an odor are enhanced and emerge earlier in terms of respiratory cycles on the background firing activity of unresponsive cells. However, the question arises: When does the bulbar pattern of an odor specifically and exclusively encode the nature of this odor?

Functional implications

Recently, different odors have been shown to produce different patterns of c-fos cRNA hybridization in topographically discrete regions of the glomerular layer and in broader, underlying portions of the granule cell layer (Guthrie and Gall, 1995). Exposure to higher concentrations of the same odor has two effects on the labeling pattern. It increases hybridization density and enhances the extension of the labeled zones, but the distribution of activated regions remains similar. The increased reactivity of bulbar neurons and the stability of response patterns reported in this study are in agreement with these results. The increasing number of responsive cells and suppressive responses may reflect respectively the enlargement of the labeled glomerular zones and the increasing extension of the periglomerular and/or granular labeled zones.

The intensity-related enhancement of the cell responsiveness observed in this study may reflect a recruitment of olfactory receptors. As previously discussed by different authors (O'Connell and Mozell, 1969; Kauer, 1974; Hornung *et al.*, 1980; Hornung and Mozell, 1981; Wellis *et al.*, 1989), the increasing OB reactivity as concentration rises probably reflects a more rapid accumulation of the stimulus molecules in the receptor sheet and a subsequent increase in the spatial size of the population of activated receptor cells (Mackay-Sim and Shaman, 1984). Moreover, the ability of M/T cells to respond to each concentration once their threshold has been reached and the constancy of their response pattern may be related to the stability of odor-induced patterns observed in the mucosa by Mackay-Sim and Shaman (1984).

Lastly, cell recruitment and response stability may be viewed as signs of an ordered evolution of the bulbar pattern of a stimulus. On the basic spatio-temporal pattern of M/T cells that responded to a stimulus, new responsive cells add progressively as intensity increases, and the spatial configuration of the bulbar message, which is pretty hazy at low intensities, becomes more distinct. However, this phenomenon has a limit, and high concentrations cannot recruit all mitral cells, thus leaving the remaining cells able to respond to other stimuli. Response constancy and the lack of massive changes in the firing frequencies of individual cells with concentration suggest that the bulbar message is a dynamic signal which evolves along the respiratory cycle, but which remains relatively stereotyped for similar epochs

of different respiratory cycles. The shift towards stimulation beginning at the maximum frequency reached by the cells during peaks may be assumed to reflect an earlier enhancement of the relief of the bulbar message on the population of unresponsive M/T cells, and thus a progressively early specification of odor characteristics. However, new studies, such as an attempt to reconstitute the OB message from the activities of individual M/T cells so as to analyze how it evolves from cycle to cycle, are necessary to validate these assumptions.

Acknowledgements

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