Mitral Cell Temporal Response Patterns Evoked by Odor Mixtures in the Rat Olfactory Bulb

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Received 29 May 2001; accepted in final form 9 April 2002

INTRODUCTION

Natural odors are often blends of several molecular components, and olfactory perception usually depends on the reception and neural processing of these components. Mixture perception has been extensively investigated psychophysically using binary or more complex odor mixtures in a number of different species, including monkeys (Laska and Hudson 1993) and humans (Laing and Wilcox 1983, 1987; Laing et al. 1984, 1994; Laing 1989; Laska and Hudson 1991, 1992; Livermore and Laing 1998). By contrast, neural representation of odor mixtures has been studied electrophysiologically only in insects, crustaceans, and fish. As a first step toward a better understanding of this processing in high vertebrates, we studied the representation of odor mixtures in the rat olfactory bulb, i.e., the second-order level of the olfactory pathways. We compared the single-unit responses of mitral cells, the main cells of the olfactory bulb, to pure odors and to their binary mixtures. Eighty-six mitral cells were recorded in anesthetized freely breathing rats stimulated with five odors and their 10 binary mixtures. The spontaneous activity and the odor-evoked responses were characterized by their temporal distribution of activity along the respiratory cycle, i.e., by cycle-triggered histograms. Ninety percent of the mixtures were found to evoke a response when at least one of their two components evoked a response. Mixture-evoked patterns were analyzed to describe the modalities of the combination of patterns evoked by the two components. In most of the cases, the mixture pattern was closely similar to one of the component patterns. This dominance of a component over the other one was related to the responsiveness of the cell to the individual components of the mixture, to the molecular nature of the stimulus, and to the coarse shape of individual response patterns. This suggests that the components of binary mixtures may be encoded simultaneously by different odor-specific temporal distributions of activity.
tained solely in the catfish (Kang and Caprio 1995). Responses were classified as excitatory, suppressive, or null depending on whether their mean firing frequency was significantly higher, significantly lower, or not significantly different from the spontaneous firing frequency. In this case, 89% of the responses to the tested binary mixtures were classified similarly as the responses to at least one of their components and were therefore predictable when responses to their two components were both classified in the same type. When two components having different response types were mixed, the mixture response type was less predictable and depended on the response types of the mixed components.

The question that we address in the present study is how mammalian mitral cell activities evoked by two single components combine in the activity evoked by their mixture. Since the odorant stimulation and consequently mitral cell odor-evoked discharges are time-locked to respiration in freely breathing mammals (Chaput and Holley 1980; Macrides and Chorover 1972; Sobel and Tank 1993), odor characteristics are supposed to be encoded in the OB by the spatio-temporal patterns of activity they evoke among these second-order neurons (Buonviso and Chaput 1990; Buonviso et al. 1992; Chaput 1986; Meredith 1986; Wilson and Leon 1987). The extensively used mean firing rate was shown not to be sensitive enough to discriminate between these patterns (Chaput and Holley 1980; Chaput et al. 1992). The activity of each cell was thus characterized in this study by its temporal organization along the respiratory cycle by means of a cycle-triggered histogram. Furthermore, previous results show that mitral cell temporal response patterns to pure chemicals were stable and reproducible (Chalanssonnet and Chaput 1998). Then, temporal patterns were utilized to define cellular responsiveness and to compare mixture-evoked responses with single odor-evoked responses. A priori, using a temporal representation, the three following types of response combination could be expected: responses to binary mixtures could be completely different from the responses of their two components, intermediate between them, or dominated by one of them. In a first step, a pattern comparison method was elaborated to decide whether two responses were identical or not. In a second step, assuming the linearity of the interaction of the components in a mixture, a linear decomposition method was used to analyze the response to a mixture as a function of the responses to its components.

METHODS

Animal preparation and cell recording

Experiments were carried out in accordance with the European Communities Council Directive of November 24th 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. Seventeen adult male Wistar rats weighing 250–450 g were utilized in this study. Animals were anesthetized with Equithesin (a mixture of pentobarbital sodium and chloral hydrate, 3 ml/kg, ip). Anesthesia was supplemented as necessary to maintain a deep level of anesthesia, as determined by the depth and rate of the respiratory rhythm of the rat and its 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.

Single-unit discharges were recorded extracellularly using glass micropipettes filled with a 2 M NaCl solution saturated with Pontamine sky blue (impedance 15–20 MΩ). Placement of electrode tips in the ventral mitral cell layer was determined by the appearance of a dipole reversal in the field potentials evoked by lateral olfactory tract stimulation and by the occurrence of large-amplitude spikes (Phillips et al. 1961). When necessary, the placement of the electrode tip was confirmed using dye spots deposited iontophoretically by passing a negative current of 2–5 μA for 15 min (10 s on, 10 s off) through the micropipette. Recordings began once a single unit had been clearly isolated. In addition to mitral-cell single-unit activity, respiratory activity was recorded through a thermistor placed just at the entrance of the nostril of the rat.

Odor stimulation

Five odorants and their 10 binary mixtures were presented for 10-s periods at intervals of ≥60 s. These five reagent-grade chemicals were acetophenone, cineole, isoamyl acetate, methyl-amyl ketone, and p-cymene, abbreviated as A, C, I, M, and P in the text. They were chosen as representative of four of the different groups (I and M belong to the same group) of the olfactory space defined in the frog olfactory epithelium (Sicard et al. 1980).

Odors were delivered with a flow dilution olfactometer described in detail elsewhere (Vigoureux and Chaput 1988). Briefly, the nozzle of the olfactometer was continuously supplied with a main flow of pure and humidified air (28 l/min). Between odor delivery, a second flow of pure air (2 l/min) was injected in this main flow. It was replaced during stimulation by an equivalent flow (2 l/min) of odorized air obtained by pumping a predetermined volume of saturated vapor from 50-l Tedlar bags using preadjusted interchangeable needle valves. This odorized flow began to be produced 10–15 s before odor delivery to allow odor concentration to stabilize in the line and it was exhausted until stimulation onset. Odor delivery was initiated 10 ms after expiration beginning, so that the first inspiration included in the stimulation corresponded to a complete stimulation period.

In the present study, we considered it crucial to equilibrate odor intensities in terms of molecular concentration, instead of equilibrating them in terms of proportion of saturated vapor pressure as done in previous studies (Chaput and Holley 1980, 1985; Chaput et al. 1992; Joerges et al. 1997; Sicard et al. 1980). Since the odorants had different vapor pressures, they were diluted differentially as shown in Table 1, so as to obtain a final partial pressure of 2.9 Pa. This concentration corresponded approximately to the dilution of 10⁻² of the saturated vapor of cineole in our previous studies, which was considered high enough to recruit most of the olfactory receptor cells responding to the delivered stimulus. Odorants were delivered singly and in mixture at the same concentration through a dynamic generation of the odor flows. Odor blends were obtained by simultaneously plugging the bags containing the two chosen odors on the injection port of the olfactometer through their ad hoc needle valves, and single components were presented by replacing the bag containing one of the two components by a bag filled with pure air. This mixture concentration was the sum of the concentrations of its individual components.

Data analysis

During experiments, signals were recorded by means of a CED-1401 Plus data acquisition system (Cambridge Electronic Design) and systematically stored for subsequent analysis. Cell activity was digitized at 15 kHz to analyze spike trains off-line. Respiration was sampled at 1 kHz and stimulation events were stored as their time of occurrence with respect to the beginning of acquisition. An example of raw data is given in Fig. 1.

Temporal pattern generation

Spikes were checked for stability and triggered using the Spike2 software (Cambridge Electronic Design). Then, the respiratory signal

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was processed to discriminate between inspiratory and expiratory phases. The spontaneous and odor-evoked spike trains recorded during the 30-s period preceding each stimulation and during the 10-s stimulation period, respectively, were represented separately as two cycle-triggered histograms. These spontaneous and odor-evoked patterns (abbreviated as SP and EP in the text) were constructed by counting the number of spikes in each of the 15 intervals (or bins) of equal duration utilized to divide each respiratory cycle, and by averaging separately the number of spikes per bin over the prestimulation and stimulation periods as exemplified in Fig. 1. Since each respiratory cycle was about 1.5 s long, the binwidth was approximately 100 ms, which has been shown to be a good compromise between precision and concision (Giraudet 2000). This type of representation could be used due to the regularity of the respiratory cycle and since the chosen binwidth was substantially greater than the imprecision in the determination of the beginnings and ends of the respiratory cycles.

Pattern comparison

Since the cycle-triggered histograms might contain a very low number of spikes per bin, they could not be compared using classical statistical methods, such as the $\chi^2$ test. A probabilistic method was thus developed to decide whether two temporal patterns were significantly different at the 0.05 level. This method considered that each cycle-triggered histogram was generated by a nonstationary Poisson process. As exemplified in Fig. 2, it was first applied to determine the responsiveness of each cell to each stimulus by comparing its EP to the mean EP, obtained by averaging its EPs recorded before the corresponding SP to determine whether the response was an overall sinusoid. Responses of a single cell to various stimuli by performing all pairwise comparisons, enough cycles ($\geq 150$) were averaged in each mean SP to fit $I_\lambda$ to SP, for each bin $i$. This allowed us to calculate an interval $I_{i,p}$ around each SP, so that if $N_i$ was generated by a Poisson process $\mathcal{P}(N_i)$, then $p(N_i \neq I_{i,p}) < p$. Any bin $i$ of the EP containing a firing frequency $N_i$ situated out of the interval $I_{i,p}$ was then considered as significantly different from the corresponding SP bin at the $p$ significance level.

By contrast, for EP-EP comparisons, too few cycles were recorded during the stimulation period to know $(\lambda_1)_{i=1...15}$ and $(\lambda_2)_{i=1...15}$, the densities of the Poisson processes that generated $N_1$ and $N_2$. Therefore we calculated for each bin $i$, the interval $I_{i,p}$ around $\lambda_1$ such that if $\lambda_1 = \lambda_2$, then $p(N_1 \neq I_{i,p}) < p$. In that case, any pair of frequencies in the same bin $(N_1, N_2)$, whose ratio was situated out of the interval $I_{i,p}$, was considered as significantly different at the $p$ significance level.

In a second step, we calculated the minimal number of pairs of bins that should be significantly different at the $p < 0.1$ and the $p < 0.01$ significance level to conclude that the two histograms were different at the 0.05 significance. Assuming that the 15 bins of each pattern were independent (Giraudet 2000), these two bin numbers, given by the binomial laws $\langle p(n = 15, p = 0.1) \rangle$ and $\langle p(n = 15, p = 0.01) \rangle$, were 5 and 2, respectively. To take into account the pairs of histograms where several bins were slightly different as well as the pairs where a few bins were very different, two histograms were considered to be significantly different whenever five bins at least were out of $I_{i,0.1}$ or two bins at least were out of $I_{i,0.01}$.

Pattern parameterization

Three parameters, for which it will be shown that no single one was sensitive enough for pattern comparisons, were defined to characterize each EP: the extensively used mean firing rate ($\langle I \rangle$), the maximum firing rate ($I_{\text{max}}$) over the 15 bins of the pattern, and the standard deviation of the firing rate around $\langle I \rangle$ (STD), which increased when the pattern was more modulated. The mean firing rate per respiratory cycle $I$ was obtained by summing the firing frequencies contained in the 15 bins of each EP. It was compared with the mean firing rate $I_{\text{sp}}$ in the corresponding SP to determine whether the response was an overall firing increase, an overall decrease, or a neutral response in terms of mean firing rate. Responses were classified as excitatory whenever $(I - I_{sp})(I + I_{sp}) > S$, as suppressive whenever $(I - I_{sp})(I + I_{sp}) < -S$,  

### Table 1. Physicochemical characteristics of the five odorants

<table>
<thead>
<tr>
<th>Odors</th>
<th>Formula</th>
<th>SVP</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetophenone</td>
<td>(A)</td>
<td>49 Pa</td>
<td>$6.0 \times 10^{-2}$</td>
</tr>
<tr>
<td>Cineole</td>
<td>(C)</td>
<td>260 Pa</td>
<td>$1.1 \times 10^{-2}$</td>
</tr>
<tr>
<td>Isoamyl acetate</td>
<td>(I)</td>
<td>728 Pa</td>
<td>$4.0 \times 10^{-3}$</td>
</tr>
<tr>
<td>Methyl amylic-ketone</td>
<td>(M)</td>
<td>490 Pa</td>
<td>$6.0 \times 10^{-3}$</td>
</tr>
<tr>
<td>$p$-Cymene</td>
<td>(P)</td>
<td>190 Pa</td>
<td>$1.5 \times 10^{-2}$</td>
</tr>
</tbody>
</table>

Saturated vapor pressures (SVP) at 25°C of the 5 odorants were obtained from the Handbook of Chemistry and Physics, 76th ed., and their dilutions are expressed in terms of proportion of SVP so as to obtain a final partial pressure of 2.9 Pa for all odorants.
and as neutral in the other cases. For consistency with the method of pattern comparison described above, the arbitrary ratio $S$ was set so that a majority (80%) of NR will be also classified as neutral in terms of mean firing rate.

**Decomposition of a mixture pattern on its components**

The simplest way to analyze the respective contribution of the patterns of the two components $X$ and $Y$ in each $XY$ mixture pattern is to determine their linear combination in the mixture. For this linear decomposition, the three patterns were considered as three 15-dimensional vectors, each coordinate corresponding to the firing rate in a bin. Then the mixture vector $\mathbf{V}_{XY}$ was projected on the plane defined by its two component vectors $\mathbf{V}_X$ and $\mathbf{V}_Y$ whenever they were not collinear. Thus $\mathbf{V}_{XY} = \alpha_X \mathbf{V}_X + \alpha_Y \mathbf{V}_Y + \mathbf{R}$, where the vector $\mathbf{R}$ orthogonal to the plane defined by $\mathbf{V}_X$ and $\mathbf{V}_Y$ was the residue of the projection, and the scalars $\alpha_X$ and $\alpha_Y$ represented the weights of components $X$ and $Y$ in the mixture pattern, respectively (Fig. 3).

Unknowns were calculated by solving the system

$$
\begin{align*}
\mathbf{V}_{XY} & = \alpha_X \mathbf{V}_X + \alpha_Y \mathbf{V}_Y + \mathbf{R} \\
\mathbf{V}_X \cdot \mathbf{R} & = 0 \\
\mathbf{V}_Y \cdot \mathbf{R} & = 0
\end{align*}
$$

FIG. 1. Examples of responses of 4 different cells obtained with 4 different odorants. Each example shows raw data and elaborate representations of the activity of the same cell recorded before, during, and after a 10-s stimulation with a pure odorant, the name of which is indicated below the stimulation pulse. Raw data comprise cell activity (Cell activ.), the respiratory signal (Respi.), and the stimulation event (Stim.). Elaborate representations of the triggered spike train consist of a raster plot, where spikes occurring during successive respiratory cycles are plotted on consecutive rows as a function of their position in the cycle, and 2 cycle-triggered histograms before (SP) and during (EP) stimulation. The corresponding mean respiratory cycle is plotted below each elaborate representation. In these 4 examples, cell activity was not synchronized or slightly synchronized (example 1) before stimulation, and odor presentation induced a synchronization of the cell activity on respiration. In example 1, the response consists of a firing increase above the spontaneous level at the end of inspiration, followed by a decrease during expiration. Example 2 presents a simple firing increase above the spontaneous level during the transition between inspiration and expiration. Example 3 shows a firing decrease during the second half of inspiration. Example 4 illustrates a more complex response characterized by a firing decrease followed by an increase during inspiration, and by another firing decrease during expiration.
Stimulus effectiveness and cell responsiveness to mixtures

The mitral cells responded on average to three pure odorants (Fig. 2 depicts a cell responsive to four odorants). Table 2 presents the effectiveness of each stimulus (i.e., the proportion of cells responsive to this stimulus) based on pattern comparisons. It reveals first a hierarchy in the tendency of five pure odorants to evoke a response. A, C, and M were the less effective and P and I were the most effective. Even in this situation where stimulus concentrations were equilibrated in terms of molecular concentration, the responsiveness to the most efficient stimulus (P) was more than two times greater than the responsiveness to the less efficient stimulus (A).

For the mixtures, this effectiveness ranged from 0.66 (for AC) to 0.84 (AI and IP), whereas the mean responsiveness to pure odorants was about 0.6 and the mean responsiveness to mixtures was 0.77. The simplest hypothesis to explain this difference was to suppose that cells responded to mixtures if and only if they responded to at least one of their components. Under this union hypothesis, the probability p(XY) of obtaining a response to the mixture XY was predicted from the probabilities p(X) and p(Y) of observing a response to X or to Y alone, assuming that these two probabilities were independent, and using the formula: p(XY) = p(X) + p(Y) - p(X)p(Y). As seen in Table 2, the predicted responsiveness was not significantly different from the observed one for all mixtures except for those containing P, which were significantly less efficient than expected. The lower efficacy of P-containing mixtures was found to be correlated with the important proportion of suppressive responses evoked by P (43%) with respect to the other odorants (20% for M and 30% for A, C, and I). This correlation might result from a higher probability of observing no response to a mixture when one of its components evoked a suppressive response.

Table 3 presents the cell responsiveness to XY mixtures as a function of the responsiveness to X and Y alone. It shows that when cells did not respond to any of the two components of the mixture, they generally did not respond to the mixture. Likewise, when they responded to one of the two components or to both, they generally responded also to the mixture. On the

**RESULTS**

A total of 430 single-odor EPs and 544 mixture EPs obtained from 86 cells were analyzed in this study. All cells were tested with the five components alone. Forty-seven were submitted further to a complete stimulation protocol comprising the 10 binary mixtures; 11 were tested with five to nine mixtures, and 14 with a few mixtures only.
whole, for 90% of the pairs, we observed a response when at least one of the two components evoked a response. This is consistent with the previous union hypothesis. However, two important differences with this model are observed. First, for 17% of 106 pairs, a response to the mixture was observed despite the lack of response to both components. This involved mainly mixtures that contained A, C, and M, the less efficient components, and could be due to an additive effect resulting from the summation of the concentrations of the two mixed stimuli. On the other hand, mixing an efficient component with an inefficient component resulted in a lack of response in 22% of 138 mixtures. This involved suppressive responses to a component in 75% of the cases, and this is consistent with the low efficacy of P-containing mixtures reported in the previous paragraph.

Pattern combination in binary mixtures

To evaluate the similarity between mixture patterns and their respective component patterns, pairwise comparisons were performed between the EP of each cell to each mixture and the EPs of its components using the method of pattern comparison. This analysis concerned a total of 544 triplets (Odor X, Odor Y, Mixture XY), 10 of them illustrated in Fig. 2. In 75% of the 268 cases in which the two component patterns were not significantly different (not shown), mixture patterns were identical to their component patterns, and therefore directly predictable. Among the 276 cases in which the two component patterns were different (Table 4), the dominance of a single component was the most represented modality of pattern combination (83%). It reached 86% in NR-R mixtures and 80% in R-R mixtures.

To analyze further how patterns combined in mixtures, the relative influence of the two component patterns in each mixture pattern was determined by applying the decomposition procedure described in the methods on the 250 mixtures for which the two component patterns were represented by non-collinear vectors (i.e., the EPs were not too similar). The resulting coefficients \( \alpha_X \) and \( \alpha_Y \) gave the relative influences of \( X \) and \( Y \) in each \( XY \) mixture. For instance, \( \alpha_X \approx 1 \) and \( \alpha_Y \approx 0 \) were representative of the dominance of \( X \) over \( Y \). As seen in Fig. 4, the different modalities of pattern combination were not clear-cut situations, but rather a continuum, with an overrepresentation of the dominance modality.

Arbitrary circular frontiers were drawn on Fig. 4 to separate the different combination modalities observed after applying the linear decomposition method. Sixty-eight percent of the pairs \( (\alpha_1, \alpha_2) \) were found in the dominance region, 18% were in the average combination region, and 14% corresponded to other modalities of combination. In 86% of cases the sum of \( \alpha_1 \) and \( \alpha_2 \) was close to 1. Binary mixture patterns were therefore close to linear-weighted averages of their component patterns, often dominated by one of them.

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**TABLE 2. Effectiveness of the 5 single odorants and their 10 binary mixtures**

<table>
<thead>
<tr>
<th>Single odorants</th>
<th>A</th>
<th>C</th>
<th>I</th>
<th>M</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effectiveness</td>
<td>0.35</td>
<td>0.49</td>
<td>0.72</td>
<td>0.57</td>
<td>0.80</td>
</tr>
</tbody>
</table>

**TABLE 3. Responsiveness of the 86 cells to 544 binary mixtures**

<table>
<thead>
<tr>
<th>Responsiveness to Pure Odorants</th>
<th>Number of Pairs</th>
<th>Responsiveness to Mixtures, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR-NR</td>
<td>106</td>
<td>83</td>
</tr>
<tr>
<td>NR-R</td>
<td>138</td>
<td>22</td>
</tr>
<tr>
<td>R-R</td>
<td>300</td>
<td>22</td>
</tr>
</tbody>
</table>

Odor-evoked activities were classified as nonrespones (NR) and responses (R). The table indicates how the cells responded to mixtures as a function of their responsiveness to the 2 components alone. Mixtures of 2 inefficient components failed to induce a response in 83% of the cases. Mixtures of 2 efficient components evoked a response in 98% and mixtures of an inefficient and an efficient component induced a response in 78% of the cases.

**TABLE 4. Percentages of dominance among the 276 different-patterned odorant mixtures**

<table>
<thead>
<tr>
<th>Pure Odorant Patterns</th>
<th>Number of Pairs</th>
<th>Dominance, %</th>
<th>Other Combinations, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR and R</td>
<td>138</td>
<td>22</td>
<td>64</td>
</tr>
<tr>
<td>Two different R</td>
<td>138</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>

One component was said to dominate in a mixture when the mixture pattern was not significantly different to the pattern of this component. As seen here, one component clearly dominated the other in 86% (22% + 64%) of the cases in NR-R mixtures and in 80% of the cases in R-R mixtures. On the whole, one component dominated the other in 83% of the cases.

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**FIG. 4. Distribution of the 2 projection coefficients \( \alpha_1 = \min (\alpha_X, \alpha_Y) \) and \( \alpha_2 = \max (\alpha_X, \alpha_Y) \) of the 250 triplets (Odor X, Odor Y, Mixture XY) for which single-odor patterns were represented by noncollinear vectors. This figure presents the different modalities of combination: independence is represented by \((\alpha_X, \alpha_Y) \approx (0,0)\), dominance is represented by \((\alpha_X, \alpha_Y) \approx (0,1)\), additive and average combination is represented by \((\alpha_X, \alpha_Y) \approx (1,1)\) and \((0.5,0.5)\). It shows that the different modalities of pattern combinations are not clear-cut situations, but rather a continuum. The peak in \((0,1)\) indicates the supremacy of the dominance effect on the other combination modalities.**
Among the 228 mixtures which components evoked different patterns and where one component dominated, we investigated the factors characterizing the dominant component in terms of responsiveness, molecular nature, and shape of its EP.

In terms of responsiveness, three situations of dominance might occur. The response to an effective component might dominate the response to another effective component (masking dominance) or the lack of response to the other component (response dominance) or it might be dominated by this lack of response (nonresponse dominance). According to the pattern comparison method, among the 138 mixtures composed of an effective and a noneffective component, 64% were dominated by the effective component and 22% by the noneffective component (Table 4). Thus in the dissymmetrical situation represented by an effective component mixed with a noneffective component, the dominance effect observed for each cell was correlated with the responsiveness of this particular cell.

In terms of molecular nature, a hierarchy in the dominance was visible among the five odorants utilized in this study. As shown by the left histogram in Fig. 5 (All cases), dominance increased from A to M components. The odorant A was the less likely to dominate, with 19% of the mixture it was involved in, and M was the most dominant with 56%. Since responses were previously shown to dominate often over nonresponses and since M, I, and P induced the highest response rates, we tested whether the cell responsiveness by itself was sufficient to explain this hierarchical order. To jointly analyze the dominance effect and the efficacy of the components to induce a response in isolation, we represented separately in Fig. 5 the cases of masking dominance (R/R) where an effective component dominated over another effective component, response dominance (R/NR) where an effective component dominated over a noneffective component, and nonresponse dominance (NR/R) where a noneffective component dominated over an effective component.

This analysis reveals that the dominance order of the five odorants observed when all situations of dominance were pooled together (All cases) was not a simple consequence of their efficacy to induce a response since it persisted when both components evoked a response (R/R). By contrast, in the last two histograms (R/NR and NR/R), this dominance order was altered by the dominance of a response over a nonresponse.

Last, the correlation between parameters extracted from the shape of the EP of each component and its capacity to dominate was analyzed. Three parameters derived from the firing frequencies in the 15 bins of the odor-evoked histogram were utilized: the mean and maximum firing frequencies of the EP ($\bar{I}$ and $I_{\text{max}}$) and an index of temporal modulation, the standard deviation (STD) of the pattern around its mean value.

As shown by the first bar in Fig. 6, the mean firing rate was not highly correlated with the efficacy of a component to dominate since only 62% of the dominant patterns had the highest mean firing rate. The maximum firing rate was more strongly involved in this domination since 69% of the dominant patterns had the highest maximum firing rate. Last, 71% of the dominant patterns had the highest STD. This relationship between dominance and STD can be partially explained knowing that most of the response patterns had a higher STD (i.e., more modulated) than the nonresponse patterns and dominated. On the contrary, nonresponse patterns were, similar to spontaneous patterns, not well synchronized with the respiratory cycle, and seldom dominated. As shown by the second histogram in Fig. 6, 73% of the dominant patterns still had the highest STD in R/R situations of dominance. Thus a modulated response was also more likely to dominate than a nonmodulated response. In NR/R situations (right histogram in Fig. 6), 80% of the NR dominating an R had a higher mean firing rate, which means that they dominated mostly suppressive responses, as seen previously. We also found (not shown) that excitatory responses dominated by an NR were significantly less excitatory than other responses, but we did not find that suppressive responses dominated by an NR were significantly less suppressive than other responses.

DISCUSSION

Prediction of mixture responsiveness and response pattern

Before the present study, no quantitative electrophysiological investigation of the responses of single olfactory bulb neurons to odor mixtures had been performed in mammals. Two fundamental results came out of this work.

I) In 90% of the cases, mixture responsiveness was predictable from the component responsiveness according to the assumption that mixtures evoked a response when at least one of their components evoked a response. Only 3% of the mixture responses could be ascribed to synergism, i.e., when mixing two inefficient stimuli resulted in a response, and 7% to sup-

![Figure 5](http://jn.physiology.org/)

**Figure 5.** Percentages of dominance of the 5 components in all situations of dominance (All cases), and separately in the cases of masking dominance (R/R), response dominance (R/NR), and nonresponse dominance (NR/R). Single odorants showed the same order of dominance in R/R situation as when all situations of dominance were pulled together (All cases).

![Percentage of dominance of each odorant](http://jn.physiology.org/)

**Percentage of dominance of each odorant**

- **A**
- **C**
- **I**
- **P**
- **M**

**Table 4**

<table>
<thead>
<tr>
<th>Odorant</th>
<th>Dominance Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R/R</td>
</tr>
<tr>
<td></td>
<td>R/NR</td>
</tr>
<tr>
<td></td>
<td>NR/R</td>
</tr>
</tbody>
</table>

![Figure 6](http://jn.physiology.org/)

**Figure 6.** Relationship between some characteristics of EP shape and the dominance effect, as measured by the percentage of dominant patterns having a higher mean firing rate ($\bar{I}$), a higher maximum firing rate ($I_{\text{max}}$), or a higher standard deviation around this mean (STD). As shown by the high $I_{\text{max}}$ and STD values obtained in all cases, dominant components generally induced a well-synchronized response pattern. Higher $I_{\text{max}}$ values in NR/R cases revealed that more than 80% of the R dominated by a NR had a lower mean firing rate than the spontaneous pattern.
pression, i.e., when no response was evoked by mixing an efficient and an inefficient stimuli or two efficient stimuli together. Thus in terms of population of activated neurons, our results support the hypothesis that the bulbar population responding to a binary mixture is quite similar to the union of the populations responding to its components.

2) In 79% of the cases, the mixture response pattern was identical to the pattern evoked by at least one of its components. When the two components evoked similar patterns, the prediction of the mixture pattern is straightforward. Otherwise, the prediction of the mixture pattern can be reduced to the two-choice prediction of the dominant component. Then, this prediction is based on the responsiveness of the cell, on the temporal organization of its response patterns to the two components, and on the nature of the stimulus. According to the combination of these parameters, the dominant pattern could be predicted with more or less certainty. For instance, a well-synchronized response to methyl-amyl ketone had a high probability of dominance over a lack of response to acetophenone.

Result 1 seems different from the conclusions of Kang and Caprio (1995) in the channel catfish since they reported that the mixture of an effective and a noneffective component was likely to be noneffective in 43% of cases. The fish bulbar population responsive to a mixture was therefore much smaller than the union of the neural populations responsive to its components. This apparent contradiction may be explained by the difference in the criteria chosen to determine the cell responsiveness (mean firing rate or temporal pattern comparison). To test this assumption, we applied in the present work the same criterion as Kang and Caprio to determine the existence of a response. By using mean firing rates instead of temporal patterns, mixtures of an effective and a noneffective component also failed to induce a response in 46% of the cases. This percentage corresponds in our study to the mixtures whose mean firing rates were not significantly different from the spontaneous firing rates, but whose temporal patterns were significantly different from the spontaneous patterns. Thus our results are consistent with those of Kang and Caprio, but the union model drawn from result 1 is only verified with our definition of the responsiveness.

By contrast, result 2 is more obviously in agreement with the conclusions of Kang and Caprio about the three-choice prediction of the type of mixture responses (Kang and Caprio 1995). Indeed, response types to binary amino acid mixtures in the catfish were generally predictable when component responses were both excitatory, both suppressive, or both null. Otherwise, the predictability depended on the mixture type. The percentage of predictability was globally the same in both studies.

Responses to different-patterned odorant mixtures

In 83% of the cases, the response pattern to a mixture of components evoking two different patterns was similar to one of these two patterns. Thus one component clearly dominated the other.

Dominated of a response over a nonresponse pattern occurred in 64% of the cases. It might simply result from the inability of the inefficient component to activate any of the receptor neurons connected to the recorded mitral cell, either directly or by way of intrabulbar neurons. Therefore this component could not influence the activity of this mitral cell, whether it was presented alone or in a mixture.

The preponderance of one response pattern over another may involve peripheral competitive or noncompetitive interactions between odorants at the receptor sites. It may also result from a difference in the time of arrival of odor molecules on different regions of the mucosa due to their transport in the inhaled air and mucus (Hahn et al. 1994; Kent et al. 1996; Keyhani et al. 1997; Mozell 1970; Mozell and Jagodowicz 1973). It may finally be ascribed to intrabulbar interactions such as lateral inhibition between mitral cells via granule cells, or to descending central influences, resulting in local suppressions of the response to one component in favor of the response to the other.

In 22% of the cases, a noneffective component masked an effective component. This possibility has been extensively shown in brain interneurons of the spiny lobster (Ache 1989; Derby and Ache 1984; Derby et al. 1985), second-order neurons of the potato beetle (Jong 1988), and olfactory bulb neurons of the channel catfish (Kang and Caprio 1995). In this
latter study, compounds that individually did not evoke a response were found to cancel the effect of excitatory as well as of suppressive components. However, when tested individually, these excitatory or suppressive components were significantly less excitatory or less suppressive than the components involved in mixtures in which one component dominated. Thus a noneffective component could mask a weakly, but not a strongly, effective component in a binary mixture. Similar results were observed in this study. Noneffective components generally failed to mask excitatory components, and when this occurred, masked only weak excitatory components. By contrast, nonresponses were often found to mask suppressive responses, either strong or weak. P and M, two of the three odorants having the highest stimulating power, were more able to dominate when they were inefficient to induce a response.

For the remaining 17% of the mixtures, none of the two components dominated, and the response pattern was either a linear combination of its component patterns or totally independent. In the latest case, the linear function may not be sufficient to take into account complex component interactions. Thus, as shown in Fig. 4, independence, composition, and dominance could not be considered clear-cut situations, but rather as a continuum. Thus even if we had no information on the spatial distribution of the recorded neurons in the mitral cell layer and cannot draw strong conclusions about the spatial characteristics of the populations engaged in mixture coding, we can conclude that binary mixtures are likely encoded at the output of the OB by the temporal characteristics specific of their individual components (Hoshino et al. 1998) and by some information more specific of the nature of the mixture contained in the responses not dominated by a single component.

We thank D. Piau (Laboratoire de probabilités, UCB-Lyon 1, Villeurbanne-France) for helpful contribution in establishing a probabilistic method to compare temporal patterns, J. W. Scott (Emory University, Atlanta, GA), and N. Buonvino (Neurosciences et Systèmes Sensoriels, UCB-Lyon 1, France) for helpful comments on the manuscript. This study was supported by the Centre National de la Recherche Scientifique, the University Claude Bernard, Lyon 1, and the Institut National Polytechnique de Grenoble.

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