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Odour discrimination by frog olfactory receptors: a second study

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Abstract. Single unit activity of olfactory neuroreceptors was recorded in frogs. Stimulations with 20 pure chemicals delivered at known concentrations elicited excitatory and/or inhibitory responses in 60 of the 76 recorded units. The responses exhibited various time patterns, partly depending on stimulus intensity. Long lasting after-effects were observed. Out of a total of 1520 odour trials, 317 excited and 33 inhibited the cells, leading to a receptor overall responsiveness of 23%. Various degrees of individual selectivity were encountered in the receptors; the greatest number responded to seven of the 20 odorants. A marked tendency to stimulate the same receptors was observed for several odorants. Three groups could be evidenced: benzene, anisole, dichlorobenzene and bromobenzene; camphor and cineole; tert-butyl alcohol, cyclohexanone and cyclohexanol. Fatty acids tended to be grouped. Sulphurous compounds elicited few responses, except thiophenol. Most of the neuroreceptors responded to odorants belonging to more than one odour group.

1. Introduction

In a previous study (Duchamp *et al.*, 1974) we developed the view that the electrophysiological responses of the olfactory receptors could offer a basis for understanding primary events underlying qualitative discrimination of odours. It was proposed that an extensive examination of the responses of many individual neuroreceptors to a large number of odorants would reveal similarities between stimulating properties, a possible basis for elaborating an objective arrangement of the odorants. Evaluating functional proximities between odorants would be a valuable starting point to identify molecular properties relevant to quality coding and to transduction mechanisms.

In the first investigation along these lines we recorded and analysed receptor unit responses to 20 chemicals covering a wide range of qualitative properties. One of the most salient features of the analysis was the demonstration that the receptors recognized as similar those compounds having an aromatic ring. It was also suggested that camphor which displayed stimulating properties markedly different from those of the so-called aromatic group could represent an other group or type.

The present paper reports the results of a second study concerning a new set of 20 odorants, some of them having been chosen according to the results of the preceding study. In addition to the data relative to qualitative discrimination, we present some aspects of olfactory receptor physiology that we evidenced in the course of the experiments.

2. Methods

The preparation and the experimental set-up have been described in detail

(Duchamp *et al.*, 1974). Decerebrated and demedullated frogs were dissected to expose the *eminentia olfactoria*, and were kept at 12°C throughout the experiments. The activity of single olfactory units was recorded by using metal-filled microelectrodes. The EOG was observed as a control of the whole responsiveness of the mucosa. The olfactory stimuli were delivered in gas phase by a multichannel olfactometer. During two seconds, a saturated vapour of an odorant was introduced at known flow rate into a permanent flow of pure nitrogen. While a single nerve unit was recorded, 20 qualitatively different stimuli were delivered in a random order at intervals of two minutes. After the series of 20 stimulations, the reproducibility of the responses was checked out by delivering again several compounds of the set. When the unit could be kept longer we used to perform additional stimulations to test various properties of the receptors, such as concentration-response functions, long-lasting after effects, fatigue or interactions between odorants.

Choice of odorants. In choosing the odorants used in the first study mentioned above we selected representatives of rather different olfactory qualities and chemical structures. The choice of a new series of compounds for the present study was guided by the following considerations: (i) to use again several odorants of the first set in order to obtain a comparison between the two series; (ii) to introduce new chemicals with an aromatic ring in order to confirm the previous indication of an aromatic group; (iii) to include some compounds chosen in the Amoore's "camphoraceous" class (Amoore, 1970) keeping in mind the particular properties of camphor as evidenced in the first study; (iv) to introduce odorants clearly related by their chemical properties, namely a series of short-chained fatty acids and a series of sulphurous compounds. Most of these substances, except sulphurous compounds, were checked for purity by means of a gas chromatograph. They are listed in Table I.

Intensity. As a rule, each stimulus was delivered at one concentration only. Before starting an experimental series we intended to adjust the injection of odour flow so that all stimuli elicited EOGs of equal amplitude (about 0.5 mV). This procedure had been followed in the first study. Unfortunately, in this series the stimulating efficiency of some compounds was so weak that the EOGs could be equalized only at a very low amplitude. Moreover, studies of the concentration-response functions of individual receptors (Holley *et al.*, 1976), performed in parallel with the quality discrimination studies, revealed two facts that guided the choice of the concentrations finally retained. The first point was that the range of odour intensity which would have allowed us to equalize the EOGs (at very low amplitude) was clearly under the threshold of many receptors. The second point was the observation that the dynamic range of the receptor transfer function appeared very short, seldom exceeding 1 log unit, and in any case much shorter than the range of intensity discrimination of the whole mucosa, as judged from EOG measurements (Ottoson, 1956, Poynder, 1974). Bearing in mind these facts it did not seem justified to realize our previous project because we would have missed most of the possible responses. On the other hand, technical and methodological limitations prevented us from systematically delivering several concentration steps of each odorant. Therefore we decided to standardize stimulus intensity in terms of constant fraction of the vapour pressure of the compounds. The concentrations were around 10 to 20% of the

Table I. Odorants used in the present study with an estimation of their concentration in the stimulating odour flow.

	Code	Chemical name	Concentration $.10^{14}$ molecules.ml^{-1}
1	BEN	Benzene	7100
2	ANI	Anisole	400
3	BRO	Bromobenzene	470
4	DIC	1,3-Dichlorobenzene	250
5	ISA	Anisaldehyde	6
6	ANE	Anethole	8
7	BZO	Benzophenone	0.5
8	CAM	dl-Camphor	35
9	CIN	Cineole	230
10	XOL	Cyclohexanol	220
11	XON	Cyclohexanone	500
12	TBU	Tert-butyl alcohol	3800
13	ABU	n-Butyric acid	130
14	VAL	n-Valeric acid	35
15	IVA	iso-Valeric acid	35
16	CAP	Caproic acid	5
17	TIO	Thiophene	960
18	PHO	Thiophenol	250
19	BOL	Butanethiol-1	-
20	SUL	Diethylsulfide	350

saturating vapour concentration of the pure odorants, except for three sulphurous compounds which were used at lower concentrations (Table I). We were aware of the fact that such a rather high intensity level could reduce the receptor selectivity and therefore strengthen the qualitative similarities as evaluated on the basis of the receptor electrical responses. Consequently, the dissimilarities or discriminations between odorants that would persist in spite of this unfavourable methodology would be all the less questionable.

Evaluation of responses. The presence of responses to stimuli was judged from visual inspection of the paper recordings keeping in mind the spontaneous patterning of the unit at rest. Some units discharged spontaneously with irregular bursts which made it difficult to appreciate whether a burst of spikes appearing during stimulation was a true excitatory response or a spontaneous discharge. However, a possible wrong estimate of the occurrence of a response or not did not entail serious consequences upon the evaluation of similarities between odorants. The responses being quantified, a "questionable" response appeared on the data table as a number not much different from the one measuring the resting frequency.

The measurement of the excitatory responses was made in terms of maximum firing frequency, assuming that such a response index feature was the one best correlated with the initial generative events evidencing the action of the odour molecules on the acceptor sites. On the other hand, an alternative way of evaluating the responses, namely the calculation of the mean frequency of firing during a fixed period of time was discarded, due to the particular response patterns of the receptor units when strongly stimulated, as discussed later. The maximum frequency was also preferred because the stimulus time course, as controlled by the use of a flame ionization detector (F.I.D.) did not have a square form, but looked like a

chromatographic peak. The maximum frequency was calculated over one second when the response was a regular and sustained discharge or over three or four interspike intervals when the response was very short with decrementing amplitude. The evaluation of the inhibitory responses took into account either the duration of the cessation of activity if any, or the mean frequency during a fixed period whenever the rate of discharge was solely decreased. The mean resting frequency was calculated over the 20 periods of 20 s. preceding the stimulations.

3. Results

During the slow penetration of the microelectrode in the olfactory epithelium, the oscillographic trace showed multiunit activity characterized by spikes with different sizes, shapes, polarities and individual firing rates. Eighty per cent of the 60 nerve units which were kept for discrimination study were found when the tip of the microelectrode was at a depth of 90 to 180 μ below the upper limit of the mucus, in the *eminentia olfactoria*. Most of them displayed triphasic spikes resembling axon spikes described by Getchell (1973). It is likely that recorded spike activity originated in fibres running in the depth of the epithelium, above or just below the basal membrane, at a level where they were not wrapped in bundles by the Schwann cells.

In agreement with the findings of the first investigation, the spontaneous activity of the nerve units was found to be low. More than 50% of the cells discharged at a frequency not exceeding 20 spikes. $min.^{-1}$

3.1 Single unit characterization

Because non equivocal characterization of a true unitary activity was required for quality discrimination studies, we paid special attention to this problem. As a rule we did not start a stimulation series when more than one unit could be detected. We renounced to use electronic devices to discriminate between several units because the relative amplitude of the spikes could change during an experiment and particularly during the response to a strong stimulus. The possible confusion between units having spikes of equal amplitude and similar shape was made minimal by respecting the following criteria: the spontaneous activity of a single unit does not usually display interspike intervals shorter than those occurring during a response to a concentrated odour puff. The occurrence of quasi-simultaneous spikes was interpreted as an indication of an additional unit. During a supra-threshold response, the spike activity becomes regular. Irregular intervals suggest mixed activity. Furthermore, the decrease in amplitude and the subsequent disappearance of the spikes which are often observed in olfactory receptors responding to a strong stimulus offer the opportunity of an additional test. If a normally-sized spike appears immediately after a decrementing response, it must be attributed to a different unit. Getting a single unit record among olfactory receptors is a time-consuming and difficult procedure, but checking the unitary character of the recording leaves no greater problem here than in other nerve tissues.

3.2 Responses

During responses to different odour stimuli, the units exhibited various discharge patterns, some of them being represented in Fig. 1 that shows the responses of unit 43 to all the odours. The arrows indicate the switching on and off of the odour

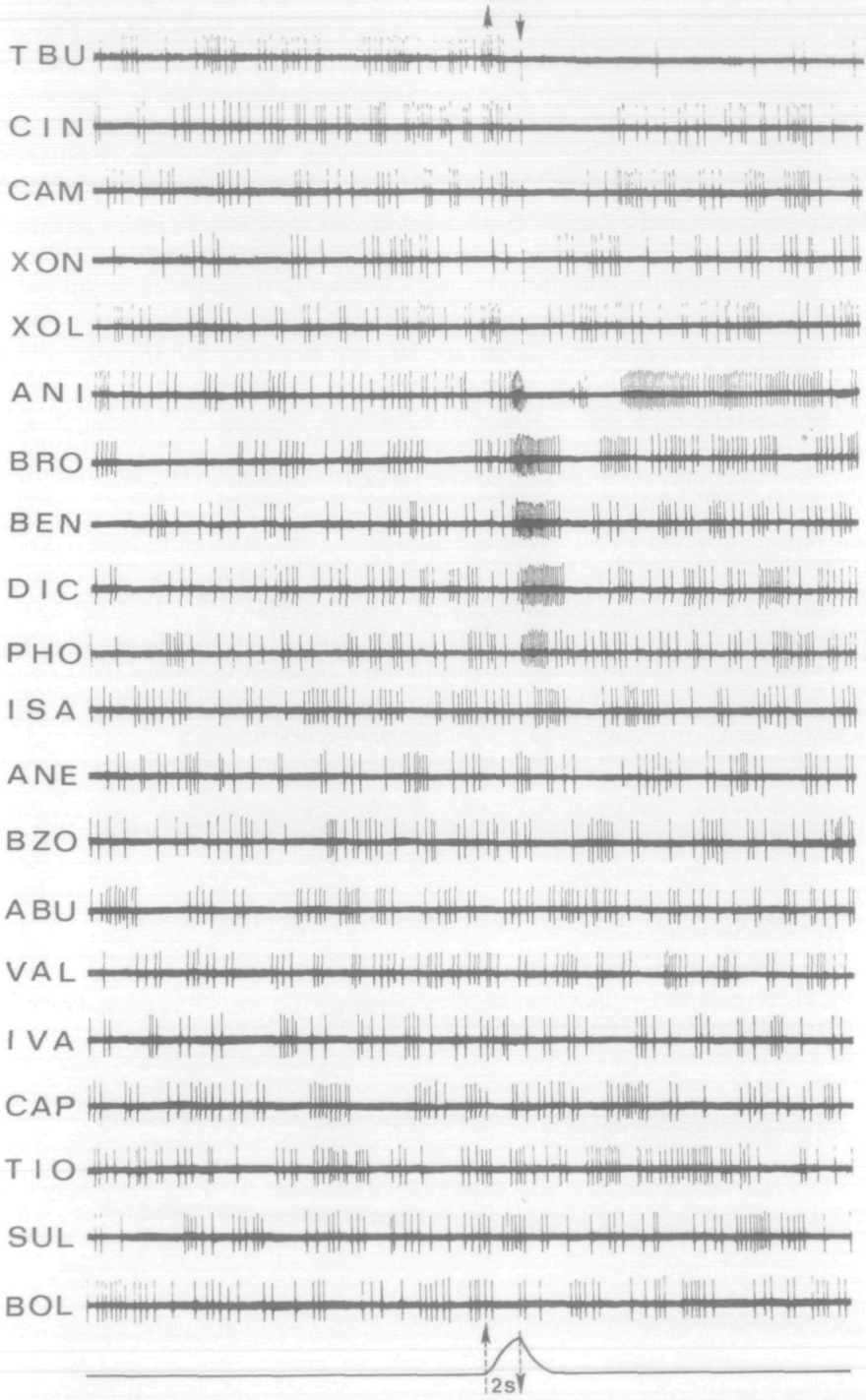

Fig.1 Electrical activity of unit 43 before, during and after stimulations with 20 different odorants. Arrows indicate odour valves switched on and off. An example of stimulus time-course monitored by F.I.D. is given.

electrovalves. As seen, the responses presented a delay. This delay included the time for the odorant to reach the mucosa (several hundreds of ms) and was specially connected with the slow rising phase of the concentration time-course. As verified by F.I.D. measurements, stimulus concentration peaked at the time when the electrovalve switched off, and then decayed during several seconds. It can be seen that the excitatory response of the unit to benzene was similar to the response to dichlorobenzene but differed from those to anisole and thiophenol. For a same odour, as exemplified in Fig. 2, the response pattern could change with concentration. As seen, a moderate concentration of cyclohexanone elicited a sustained discharge. When we applied higher concentration, the discharges had a higher frequency and the latency diminished. It can also be seen how the silent period after the initial burst augmented. At higher concentrations, the response could be limited to three or four spikes with very short interspike intervals. It was consistently observed that this short discharge was decremential, the spikes undergoing a drastic reduction of their size and disappearing in the background noise. The maximum frequency never exceeded 20 spikes. s^{-1}.

In several instances, one of which is shown in Fig. 3, when the unit displayed a high signal-to-noise ratio, the spikes did not disappear but could be seen, just overtopping the baseline noise. These instances suggest that the sudden disappearance of the spikes following a strong excitation is not indicative of an actual secondary inhibition but could represent marked changes in the generative or conductive properties of the receptors. The point is of importance, as far as the quantitative estimation of the responses is concerned, and will be discussed further.

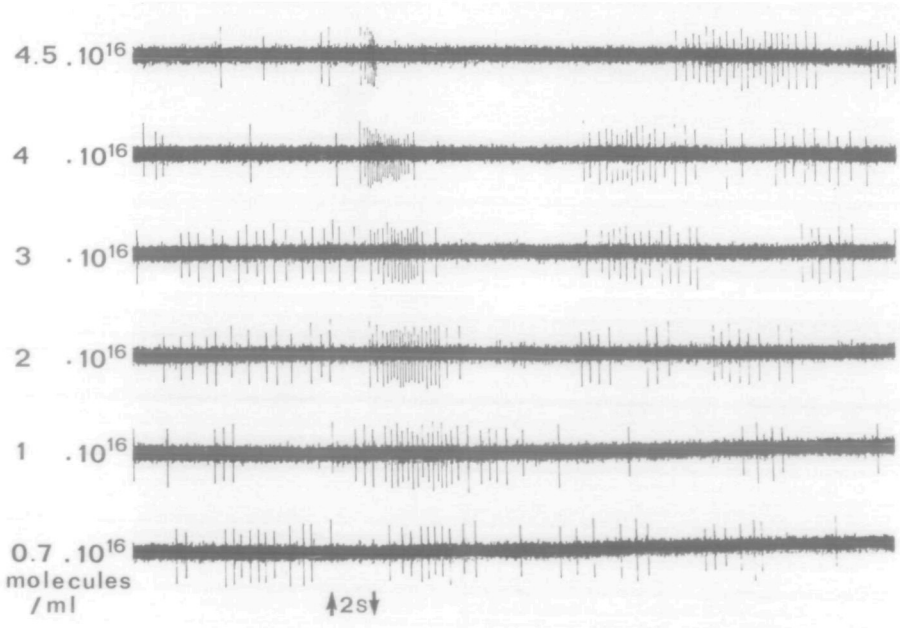

Fig.2 Response patterns of a receptor (unit 34) stimulated with different concentrations of cyclohexanone.

As seen in Fig. 4, it happened that strong stimulations induced rhythmical bursts of spikes for minutes. It was most often observed with camphor, cineole, tert-butyl alcohol and cyclohexanone. If the spikes had undergone a reduction in size as a consequence of excitation, the size increased progressively in course of the successive bursts.

Inhibitory responses appeared as a marked reduction or a complete arrest of spike activity for several seconds. The responses of unit 43 displayed in Fig. 1 illustrate various degrees of inhibition to tert-butyl alcohol, cineole, and possibly camphor.

3.3 Effect of previous stimulation

A special attention was paid to possible effects of previous exposure on subsequent responses to different stimuli. Although such stimulus interactions were unusual, they were suspected several times and clearly evidenced in two units in which they could be thoroughly investigated. It appeared that the response to an odorant was modified by a previous stimulus delivered two minutes sooner.

A first instance is represented by cell 16 which was excited by cineole and dichlorobenzene. As seen in Fig. 5, the response of this cell to dichlorobenzene was

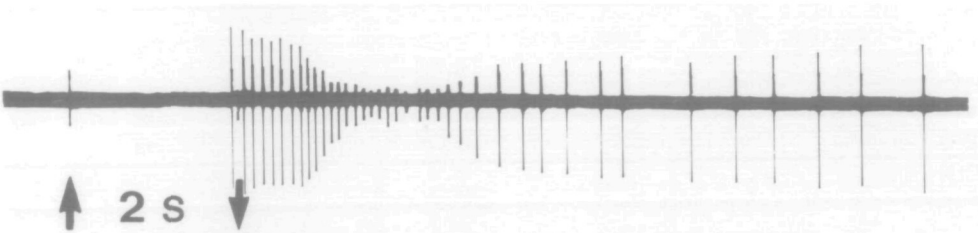

Fig.3 An example of response to cyclohexanone showing a decrement of spike amplitude (unit 21).

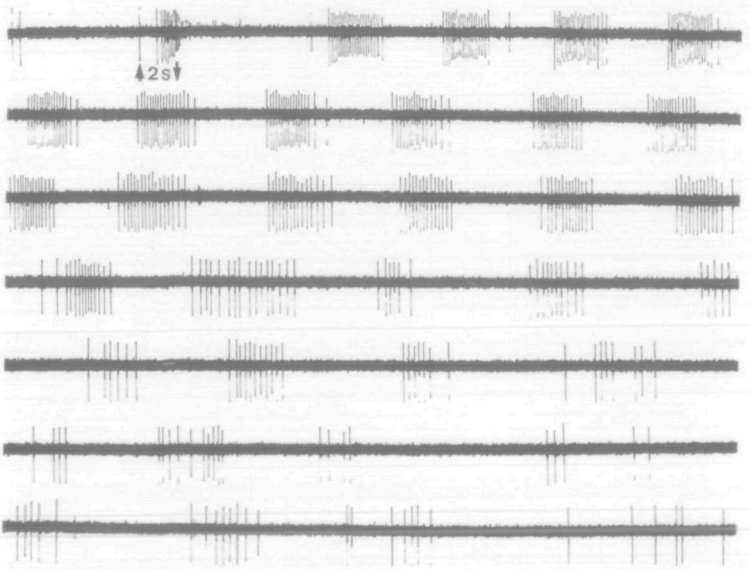

Fig.4 An example of long-lasting after effect occurring after a strong stimulation with cyclohexanone (unit 38).

completely inhibited by previous stimulation with cineole. The suppressive effect of cineole upon dichlorobenzene response was still observed when the adaptive stimulus was applied six minutes before the test stimulus. Cineole also reduced, but did not obliterate the response to benzene. The interaction was not reciprocal as neither benzene nor dichlorobenzene could be found to affect the discharge evoked by cineole.

A more complex instance of interaction between stimulations was observed in cell 15 to which cineole elicited either an excitation or an inhibition, depending on the nature of the stimulus previously delivered. As shown in Fig. 6, when no stimulus had been delivered during several minutes, or when a preceding stimulus had elicited no response, cineole induced a moderate, excitatory response. This observation could be repeated nine times. Conversely, when cineole had been delivered two minutes after a stimulation with anisole, bromobenzene,

Fig.5 An example of interaction between two odour stimuli. The response of unit 16 to dichlorobenzene was prevented by a previous stimulation with cineole.

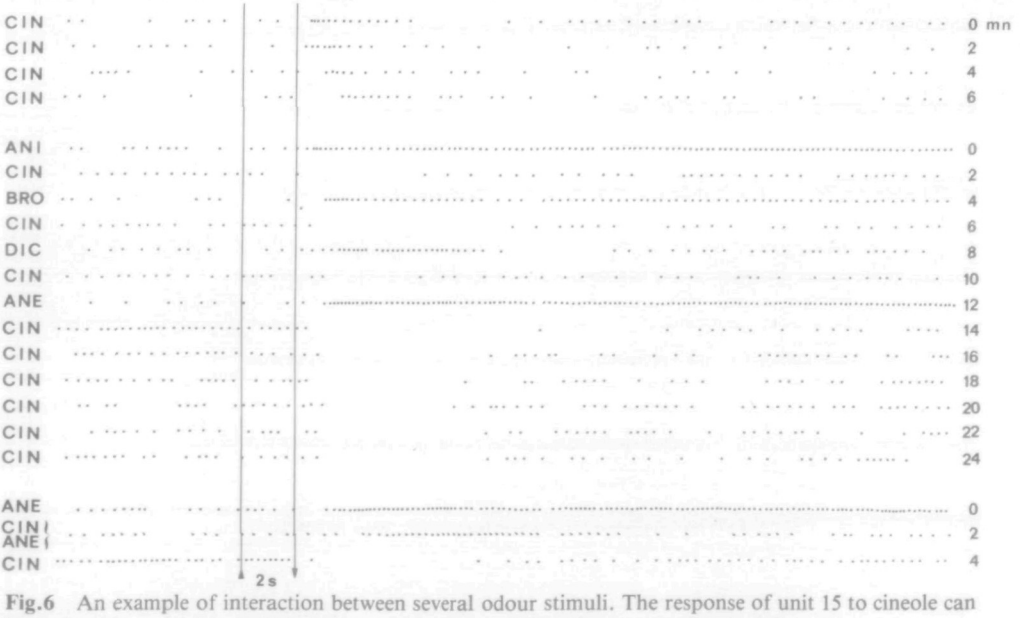

Fig.6 An example of interaction between several odour stimuli. The response of unit 15 to cineole can be excitatory or inhibitory depending on the nature of the stimulus previously delivered.

dichlorobenzene or anethole, the response to cineole was clearly inhibitory (20 observations). This inhibition was all the more easily observed as some "adaptive" stimulus, such as anethole, elicited a long-lasting excitation. However, inhibition could not be considered as being a simple interruption of the previous excitation as the firing frequency fell down under its resting level. It was further observed that a stimulation with a mixture of cineole and anethole induced a weaker response than the response to pure anethole. However, when cineole was used as an adaptive stimulus, it did not affect the response to any excitatory odorants subsequently delivered.

3.4 Odour discrimination

Out of a total of 1520 odour trials given to 76 units, 317 excited and 33 inhibited the cells, thus the receptor overall responsiveness was 23%. Sixteen receptor units failed to respond to any stimulus. The responsive receptors showed various degrees of individual selectivity. As shown in Fig. 7, a larger number of receptors responded to seven of the 20 stimuli. The fact that excitatory responses greatly prevailed over the inhibitory ones may be partly accounted for by the difficulty of evidencing inhibition when the spontaneous activity was low. All the odorants having elicited inhibitory responses also elicited excitation in other cells. Unit 43 shown in Fig. 1 exemplifies the fact that a unit could be excited by some odorants and inhibited by others. Only three units were purely inhibited.

The 60 receptor cells that exhibited unambiguous unitary activity throughout a stimulation series and gave at least one response to the whole set of stimuli, were

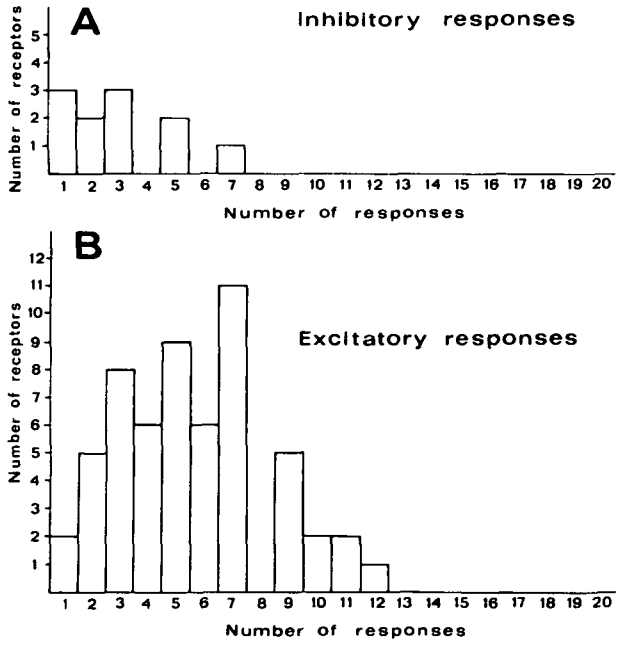

Fig.7 Histograms showing the distribution of the neuroreceptors as a function of the number of inhibitory (A) and excitatory (B) responses they displayed to 20 different stimuli.

selected to investigate similarities between odorants on the basis of their response spectra.

Figure 8 symbolically represents the table of numerical data. In the diagram, the area of each dot is roughly proportional to the firing frequency of a receptor unit stimulated by an odour stimulus. For a receptor, along a horizontal line, equally-sized spots correspond to resting frequency (no response), larger spots, to excitatory responses, and smaller spots to inhibitory responses. In this table, receptor units and

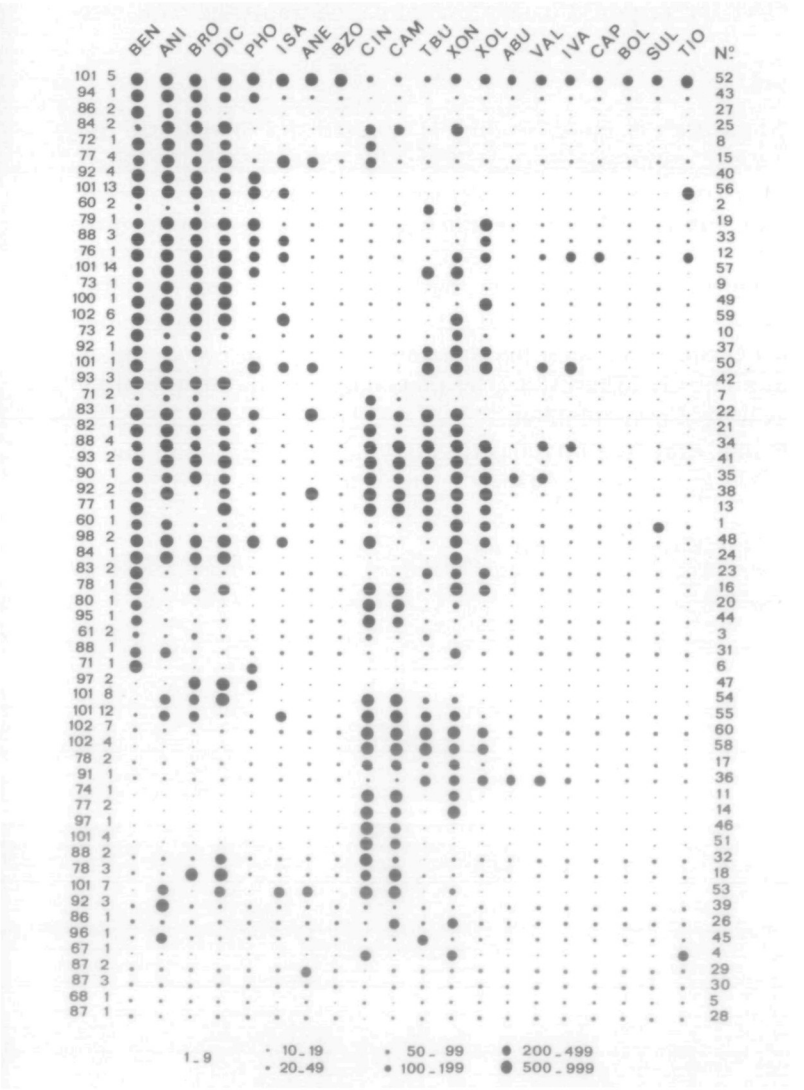

Fig.8 Diagrammatic representation of the spike frequencies (spikes. min^{-1}) that were used in quantitative evaluation of odour discrimination. The area of the circles is approximately proportional to spike frequency. Along a line a response appears as a larger spot (excitation) or a smaller spot (inhibition) with respect to the equally-sized spots representing the resting frequency of a nerve unit. Lines and columns were arranged in order to disclose patterns. Receptors are identified by the number of the frog followed by the number of the unit (left column) and by a serial number (right column).

stimuli have been ordered as to demonstrate the most obvious features of the results. Without any further analysis it is easily observed that benzene, anisole, bromobenzene and dichlorobenzene tend to elicit responses from common receptors, and the same can be said about cineole and camphor. Moreover, it is obvious that the two populations of receptors responding respectively to the aromatic compounds and to the camphor-cineole pair overlap to some extent. On the other hand, tert-butyl alcohol, cyclohexanol and cyclohexanone that have a marked tendency to be associated in the receptor response spectra, are also perceived by a large part of the receptor set responding to the camphor-cineole pair. It can also be observed that no receptor responded to only one of the four fatty acids. The acids show no preferential association with any other group of odorants. Sulphurous compounds elicited very few responses. The most efficient among them, thiophenol, seems to elicit responses from those receptors mainly responding to aromatic stimuli. The weak efficiency of the sulphurous compounds was reflected in the EOG measurements. Stimulations with thiophene, butanethiol and diethylsulfide failed to elicit any measurable EOG in most of the frogs while the EOG for thiophenol was around 0.2 mV.

4. Discussion

The results of this study present two main aspects that will be successively discussed. The first aspect concerns some general properties of the neuroreceptor activity at rest and during odour stimulation. In discussing these results, our main concern will be to enlighten the context of the discrimination study and to justify the criteria chosen to classify and quantify the unit responses. The second part of the study deals with the discrimination itself. The related comments will be limited to the most conspicuous results of the experiments. A statistical analysis of the same data and more complete theoretical considerations will be presented in a subsequent paper (Revial *et al.*, 1977).

4.1 General properties of the nerve units

The results of the present study are in good agreement with those of the previous work (Duchamp *et al.*, 1974) as far as several general properties of the olfactory receptor units are concerned: low spontaneous rate of firing, existence of units not responding to a set of 20 chemical stimuli, relatively low rate of firing during the responses, presence of responses with typical amplitude decrement and appearance of rhythmical bursts of spikes following strong stimulations. Concerning the spontaneous activity, it was not possible to find out any relationship between the spontaneous firing level of a unit and any property related to stimulus discrimination. It was only observed a marked tendency of the units with the lowest resting frequencies to reach the highest firing rates during the responses. On the other hand, the fact that inhibitory responses were exclusively observed in those units displaying rather high spontaneous firing level may purely result from the obvious difficulty in observing a decrease in activity in almost silent units. However it cannot be inferred that inhibition and excitation are evenly distributed in the responses of the units that have a weak spontaneous activity. On the contrary, excitation seems more frequent than inhibition. For instance, let us consider stimuli such as aromatic compounds, cineole and cyclohexanol, each of them excited more than 50% of the tested units.

In addition, it is clear that inhibitory responses, if present, could have been detected in about 50% of the remaining units that displayed a moderate activity. Even if one assumes that the silent units with the so-called "no responses" were in fact inhibitory ones, the actual ratio of inhibitory responses to some stimuli could not exceed presumably 25%.

In connection with the problem of spontaneous activity, one may question whether our way of collecting units results in a good sampling of the receptor cell population since units that do not emit spikes spontaneously are not included in the sampling. In recent experiments (Juge and Holley, 1977), DC polarization of the olfactory epithelium was used to reveal these silent units. In many instances, units were discovered which had no resting activity and did not respond to the mechanical stimulation caused by the microelectrode penetrating slowly in the tissue. It remains to know whether these cells display receptive properties similar to those of the spontaneously active cells.

The 16 cells that failed to respond to any odorant can be thought of as being either damaged cells, injured by the electrode, non olfactory cells, or receptor cells lacking adequate membrane receptor equipment. A choice of other odorants could have excited these cells. Analysis of spike voltage configuration (Getchell, 1973) did not give support to the assumption of damaged cells.

The relatively low rate of firing reached by the units in the course of the responses, even at high stimulus concentration, is in agreement with previous findings (Gesteland *et al.*, 1963, 1965). It must be put together with the drastic shortening of the responses and the spike decrement occurring at these high levels of stimulation. These phenomena have been interpreted in terms of variations in the ionic content of extracellular and intracellular spaces along with active transport processes inadequate to repolarize the cell (Holley *et al.*, 1974, Gesteland, 1976). However, there is no evidence to support this assumption. It is hardly conceivable that the emission of three or four action potentials, as it happens at high concentration, suffice to seriously modify the ionic gradient across the axonal membrane. What is more, in DC polarization experiments on the neuro-epithelium (Juge and Holley, 1977), it was demonstrated that spike reduction in response to odour could occur without previous firing of normally-sized spikes. Spike decrement and discharge shortening seem to implicate an alteration of the spike generative mechanisms. An overdepolarization caused by strong stimuli could have such consequences, specially if one assumes that the generator potential spreads electrotonically in the axon at a certain distance from the soma, up to the recording site. According to this hypothesis, it is possible that the spike electrogenesis is interrupted before the generator potential has reached its maximum. If so, the spike frequency would be more directly related to the initial slope of the generator potential than to its maximum value. Accordingly, an evaluation of the olfactory responses in terms of maximal rate of firing seems more relevant to primary molecular events than any other criteria, such as total number of spikes in the response or discharge duration, at least with high stimulus intensity.

It was previously reported (Duchamp *et al.*, 1974) that high level stimulation intensities sometimes evoked typical patterns of regular and rhythmical bursts of spikes in some units. Several identical observations could be repeated in the course

of the present study. Although this type of activity was not observed with all the odorants, but only for the most efficient of them, it seems unreasonable to assume that it could be implicated in quality discrimination. More probably, this phenomenon reflects an unspecific reaction of the neuroreceptors to an excessive stimulation. Nevertheless, it should be of interest to investigate further this aspect of the neuroreceptor physiology.

The mean receptor responsiveness (23%) of 60 cells is close to that (18.5%) found in our previous study. The distribution of the receptor cells with respect to the number of odorants to which they responded shows a markedly different pattern. A great number of cells responded to seven stimuli instead of one or two in the previous study. An explanation for this discrepancy can be searched in an increased stimulus concentration. However, this would also implicate an increase in the mean receptor responsiveness, which was hardly observed. More presumably, the difference comes from the qualitative change in the stimulus set.

4.2 Odour discrimination

The pattern emerging from the arrangement of the receptor responses, as seen in Fig. 8, deserves several comments. First of all it appears that the clear-cut pattern resulting from a quantitative estimate of the responses does not greatly differ from the one which could be built in binary terms, i.e., presence of a response or not. Obviously this result is related to the high level of stimulation intensity. Most of the responses reached a value that is close to the maximum rate of firing of the units. The fact that few responses had middle frequencies seems to indicate that the sensitivity spectrum of each receptor, although wide, is rather sharply defined. Increasing odour intensity does not suffice to cause any receptor to respond to any odorant. What is observed is precisely what could be anticipated if receptor cells possessed defined and discrete "acceptors", the term being used for convenience without any assumption about underlying mechanism. Olfactory stimulation at high intensity level could be favorable to an experimental approach of the qualitative aspects of olfactory discrimination fairly well separated from the intensitive aspects.

Among the 20 stimuli, three groups involving nine odorants can be recognized on the basis of their systematic tendency to excite the same units. The first group includes benzene, anisole, bromobenzene and dichlorobenzene. The second is the cineole-camphor pair. The third group includes tert-butyl alcohol, cyclohexanone and cyclohexanol. It must be remembered that in our first study (Duchamp *et al.*, 1974) benzene and anisole had been found to be included in an aromatic group with four other benzene derivatives. The existence of this group is confirmed with this additional involvement of two new compounds. The confusion made repeatedly by the receptor units between camphor and cineole indicates these compounds use common membrane acceptor mechanisms. The third group of odorants had not been previously identified.

The data table calls for another remark. Most of the units respond to stimuli belonging to more than one of the previously defined odour groups. It follows that the receptor classification does not parallel the odour classification.

Several odorants showed themselves weak or ineffective stimuli for the frog receptors: benzophenone, sulphurous compounds with the exception of thiophenol and

fatty acids, specially caproic acid. Concerning fatty acids, the results agree with those of the preceding study in which butyric acid elicited few responses. On the other hand it is rather surprising that sulphurous compounds that are powerful stimulants for man failed to stimulate the frog neuroreceptors. One of them, butane-thiol, was diluted in liquid phase and its actual vapour concentration was not evaluated. However it remained well above the perception threshold for the experimenters. A special attention was paid to the possible toxic effects of these compounds that could be expected to have a paralysing action on the olfactory epithelium. However we never observed any sign of activity blocking during stimulation or interaction with other odorants. To explain the weak stimulating power of S and S-H compounds, one may suppose the frog neuroreceptors do not possess acceptor sites liable to recognize S or S-H groups as such. In this connection, it is worth noting that thiophenol, whose stimulating efficiency contrasted with that of butane-thiol, elicited responses exclusively from those units also responding to benzene. It could get its property from its benzene nucleus, the S-H group being unoperant. Another hypothesis could be proposed: in the frog, and possibly in other vertebrates, the acceptor sites for S or S-H groups could be distributed in a small number of specialized receptor cells so that the probability of recording specific responses would be very low.

In two cases it was observed that the response of a receptor unit to a stimulus had been modified by a previous stimulation. Cross-adaptation mechanisms, such as those studied by Baylin and Moulton (1977) in the receptor cells of the tiger Salamander, might explain these observations. The findings suggest that unit 16 has two different acceptor mechanisms that are able to recognize cineole. One would also recognize dichlorobenzene and its function would be adapted by cineole and dichlorobenzene. A second acceptor mechanism, specific for cineole, would explain the non-reciprocal character of cross-adaptation. In the example of unit 15 that was either excited or inhibited by cineole depending on the preceding stimulation, the existence of two different cineole acceptors could be further proposed. One of them, excitatory in nature, would be common to both cineole and aromatic compounds, and would be responsible for the excitatory response to cineole. Following cross-adaptation with an aromatic compound, the inhibitory response to cineole would depend upon the function of a second type of acceptor, specific for cineole, and inhibitory in nature. The distribution of aromatic and camphoraceous compounds in two different odour groups suggests that these two categories of stimuli act upon different molecular recognizing mechanisms in the receptor cells. As it will appear in the discussion of the companion paper, this does not contradict the view that a certain type of acceptor is activated by cineole and by aromatic compounds.

Acknowledgements

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