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UNITARY RECORDINGS OF NEAR THRESHOLD RESPONSES OF RECEPTOR CELLS IN THE OLFACTORY MUCOSA OF THE FROG

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SUMMARY

1. Receptor cell activity in the frog's eminentia olfactoria was recorded using metal-filled micro-electrodes.

2. Several units discharged spontaneously with a mean frequency lower than 0·2 spikes per sec, or were silent in periods of up to 5 min. The other units displayed spontaneous activities between 0·2 and 1·05 spikes per sec; their activity could be modelled with a Poisson process.

3. Near-threshold responses to odour stimulation were investigated, considering several stimulations within a small concentration range. Low concentration stimulations were sometimes followed by a response, sometimes not. The concept of response probability is introduced to describe this incertitude.

4. The distribution of the number of spikes in several odour trials at low concentrations showed a reasonable agreement with two types of Poisson distribution.

5. The findings are discussed in connexion with receptor cell sensitivity and the excitation of second order neurones in the bulb.

INTRODUCTION

The convergence of numerous olfactory receptor cells to a much smaller number of secondary neurones is generally recognized (Allison & Warwick, 1949). Such a system results in an 'amplification' of the responses to natural stimuli, visualized for example by imagining 1000 receptors each responding with one spike within 1 sec after presentation of a stimulus. The impact on the connected secondary neurones would be considerable (van Drongelen, Holley & Døving, 1978; Holley & Døving, 1978). That is to say, a response in the secondary neurones might be evoked at a much lower concentration than is commonly used to study activity of single receptors. Previous description of receptor cell activity have been made from moderate to high concentrations of stimuli (O'Connel & Mozell, 1969; Gesteland, 1976; Getchell, 1974; Duchamp, Revial, Holley & MacLeod, 1974; Revial, Duchamp & Holley, 1977). Upon stimulation with low concentrations, receptor cell responses might be evident as an occurrence of spikes or a modified spontaneous discharge. These events may represent spontaneous fluctuations or be true responses. Thus at lower concentrations conventional description of the stimulus–response relation fails.

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This paper aims to describe the stimulus–response relation around the so-called threshold concentration. One can study the effect of single cell stimulation at a low concentration in a large number of receptor cells, or alternatively regard the effect of several such stimulations on one single cell. In the present study the latter procedure has been chosen. Statistical methods have been used to evaluate the fluctuations in response at low stimulus intensities, and to relate receptor cell response properties to stimulus concentration. A short description of this work has appeared previously (van Drongelen, 1978).

METHODS

Preparation technique. In the present experiments twenty-two frogs of the species *Rana ridibunda* and *Rana esculenta* were used. The preparation technique was the same as used by Duchamp *et al.* (1974). The frogs were spinalized, fastened to an operating stand and the eminentia olfactoria were exposed by removing the dorsal surface of the nasal cavity. The exposed eminentia olfactoria were observed under a stereo microscope. A preparation was rejected when the olfactory epithelium was unpigmented, or the mucus seemed too abundant or ropy. After surgery, the frog was covered with wet gauze and put on a small platform fixed to a heavy block preventing vibration. The platform on which the frog was attached was kept at 13 °C throughout the experiment.

Stimulus selection. In the present study four odours were used: butanol, isoamyl acetate, cineole, and anisole. The choice of odorants secured a high probability of eliciting a response, as they have been shown to be very effective stimulants for frog olfactory neuroreceptors (Duchamp *et al.* 1974; Revial *et al.* 1977). Using these odorants, a comparison of the findings with those of previous studies was possible.

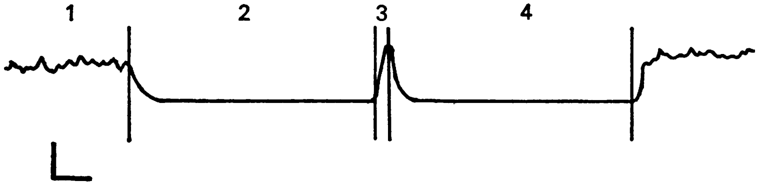

Fig. 1. The flame ionization signal during a recording of neural activity. Stimulant: cineole. Calibration bars indicate 3 sec and a fraction 1/3200 of the saturated cineole vapour pressure. The four steps separated by the vertical bars indicate: step 1, during which spontaneous discharge was recorded; steps 2 and 4, during which the mucosa was exposed to a N_2 flow; and step 3, during which odorant was injected in the N_2 flow.

Stimulating apparatus. The odour stimuli were administered by an olfactometer permitting delivery of different odour stimuli at eight concentration steps ranging from 1/5th to 1/5000th of the saturated vapour pressure of the pure chemicals. This range of concentrations was obtained by combining liquid-phase and gas-phase dilutions. Diluents used were paraffin oil and purified N_2.

Stimulus concentration monitoring. To measure the stimulus concentration and to determine its time course, a flame ionization detector (F.I.D., Becker 741) was used (Bostock & Poynder, 1972; Poynder, 1973; Holley, Delaleu, Revial & Juge, 1976). A short probe tube connected into the detector continuously transported a sample of the odour to be tested into the H_2/O_2 flame. A N_2 stream, flowing in a smaller tube hooked into the open end of the probe, created a negative pressure which withdrew the odour sample and transported it quickly into the flame. To obtain reliable measurements for the stimulus concentration, the probe was placed immediately beside the odorant flux and as close as possible to the recording site on the mucosa. The signal of the flame ionization detector was recorded on a potentiometric recorder.

One recording sequence of unitary activity consisted of the following four steps: step 1, in which spontaneous activity was recorded (15 sec); step 2, in which the mucosa was exposed to a purified humidified N_2 flow of 4 ml./sec (20 sec); step 3, a stimulation step during which a puff of odour vapour was injected at 1 ml./sec into the N_2 flow (1–2 sec); and step 4, an after-stimulation step with a purified and humidified N_2 flow (20 sec). Throughout the experiments a fan withdrew the vapours. An example of flame ionization signals during one odour trial is shown in Fig. 1. In this Figure the four steps described above are clearly visible. In step 1 a fluctuating signal due to room pollution can be seen; during N_2 flow (steps 2 and 4) the signal is stable. The amplitude of the signal in step 3 has been taken as a quantitative measure for the stimulus strength. It should be noted that the odorant reached the olfactory epithelium with some delay after injection of odour vapour in the N_2 flow.

Recording of neural activity. Single receptor responses were recorded using metal-filled electrodes described by Gesteland, Howland, Lettvin & Pitts (1959). The tips were covered with a gelatinized platinum black sponge. The diameter of the platinum sponge was 3–5 μm. The impedance of the micro-electrodes was between 0·5 and 2 MΩ measured at 1 kHz. The micro-electrode was connected to a conventional high gain amplifier of band pass 0·3–3 kHz, and 20 MΩ input resistance. An Ag/AgCl electrode, embedded in a cotton pad soaked with Ringer solution and placed in the frog's mouth, served as reference electrode. Spikes were displayed on an oscilloscope and recorded on a magnetic tape for subsequent analysis. Selected neural activity was displayed by means of a U.V. recorder.

Procedures. Zero depth of the electrode tip was determined by the electrical artifact accompanying contact of the micro-electrode with the mucus. Unitary activity was recognized by visual examination of the action potentials on the oscilloscope screen, and by listening to the auditory monitor. In multi-unit recordings it was sometimes possible to recognize a single unit of activity by discrimination of spike amplitude. If a difference less than a factor of two in spike amplitude between the units was displayed the activity was rejected. A window discriminator to extract unitary activity from multi-unit recordings was not used.

When single unit activity appeared, low concentration stimulations were given to evoke a response. In many cases the neural activity was rejected because several units responded to the odour. After stimulus onset sometimes spike activity of units that did not display spontaneous discharge was encountered, so that this type of unit could be isolated. Units that were searched for apparent threshold concentration did not show any of the following properties: (1) a signal-to-noise ratio lower than two, (2) a rise of neural activity accompanying electrical artifacts, (3) large variations in spike amplitudes, (4) clearly visible responses to the purified N_2 flow preceding olfactory stimulation. After determination of the apparent threshold concentration, several odour trials (five to sixty) below this concentration were given (sub-threshold stimulation). In a series of recordings of near threshold activity, an interstimulation time of 55 sec was used. In addition, in twelve units a few responses above threshold level (superthreshold stimulation) were recorded to complete a series. The successive measurements of these responses were performed on stepwise increases of the stimulus concentration, with an interstimulation time of 115 sec.

Statistical methods. Spontaneous activity of the selected units was modelled by a Poisson process. A specific characteristic of a Poisson process is that the mean of the intervals equals its standard deviation (Cox, 1962). Intervals were measured for units that displayed at least 101 spikes and their mean and standard deviations were calculated. In units that displayed a low spontaneous activity, the number of spikes in the response was counted for each near threshold odour trial. From these figures, the mean number of spikes and their variance were calculated. These parameters were used to analyse the distribution of spike numbers for the different odour trials with two types of Poisson distribution (Moroney, 1956). One may expect that the responses are taken from one and the same Poisson distribution if the stimulus concentration is exactly the same for each odour trial. During the expts. the stimulus concentration varied slightly from one odour trial to another. Because the mean number of spikes in a series of responses is probably a function of stimulus concentration, even within a small concentration range (as during the expts.), the responses may be expected to be taken from different Poisson distributions. For this reason a Poisson distribution modified for the variance was used. When theoretical distributions were applied to describe recorded values, a chi-square test was used to judge goodness of fit (Moroney, 1956).

RESULTS

The posterio-lateral part of the left eminentia olfactoria was searched for neural activity. The electrode entered the epithelium at an oblique angle. When the electrode tip had penetrated between 40 and 250 μm, spike discharges from one or several units were encountered. The present study is based upon the results from twenty-seven units selected, as described above. The signal-to-noise ratio of these units was between 2 and 6.

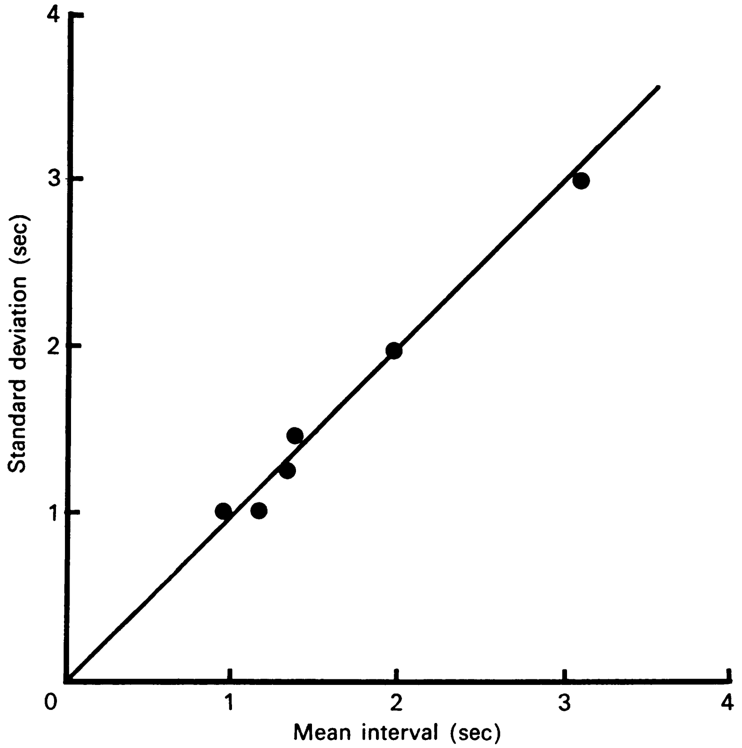

Fig. 2. Relation between the mean and standard deviation of interspike intervals of six units (class 2). The line represents the theoretical values predicted by a Poisson process.

Spontaneous activity

Most of the receptor cells displayed a continuous discharge of spikes in the absence of overt olfactory stimulation. Some of the selected units (nine) displayed no spontaneous activity in an observation time of 5 min or more. The spontaneous activity of the other units varied from 0·03 to 1·05 spikes per sec. The units were separated into two classes on the basis of their spontaneous discharge rate. In twenty-one cells the spontaneous activity was below 0·20 spikes per sec (class 1). Only six cells were found that had a spontaneous activity above 0·20 spikes per sec (class 2). This separation was made because it was unpractical to use the same kind of analysis of the activity of units of the two classes.

The mean and standard deviations of interspike intervals for the six units of class 2 were calculated and the result is shown in Fig. 2. In this diagram the expected values predicted by a Poisson process (line) are indicated. The goodness of fit of the observed data to the expected values is evident. A similar finding for units that display a spontaneous activity with mean intervals ranging from 0·07 to 4 sec has been reported by Getchel (1974).

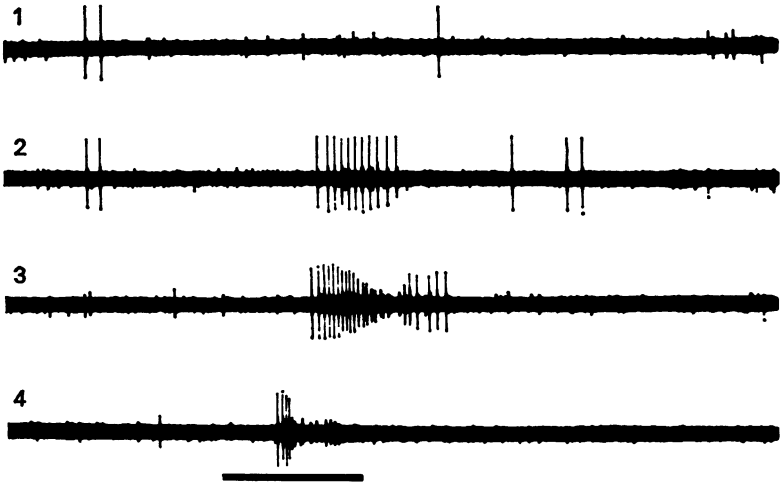

Fig. 3. Unit 24. Stimulant: isoamyl acetate. Trace 1, a weak response?; traces 2, 3 and 4, responses at super-threshold stimulations. The stimulus concentrations measured by the flame ionization detector were 2·15, 11·00, 14·40 and 80·00, for the traces 1, 2, 3 and 4 respectively. The concentration units represent 1/6092 of the saturated isoamyl acetate vapour pressure at 20 °C. (This figure has been slightly retouched.)

Responses to odour stimulation

High concentration. When an odour puff was given to the olfactory mucosa a change in the spontaneous discharge frequency, distinguishable from the spontaneous background activity by auditory and/or visual means, was sometimes observed. Receptor cell responses to stimulations above a certain concentration were easily recognized using this criterion. Furthermore, very strong responses were always characterized by a decrement in spike amplitudes. Examples of the different effects evoked by odour stimulations of various concentrations are shown in Fig. 3.

A quantitative measure of the response might be obtained by determining for a fixed time interval the number of spikes, the mean spike frequency, or the maximal spike frequency (inverse of the smallest interval, Revial *et al.* 1977). In Fig. 4 an example of the relation between the stimulus strength and the response in terms of maximal spike frequency is shown. In this Figure it can be seen that lowering the stimulus concentration caused the maximal frequency of the response to diminish to a certain value T. At lower concentrations conspicuous responses were not obtained. The value T might be called the receptor cell threshold.

Low concentration. To study the effect of odour trials at low concentrations several stimulations were made. For units of low spontaneous activity (class 1), the number of spikes occurring during the 22 sec after the onset of stimulation served as an index of the response. By using this duration all possible fluctuations of subthreshold ought to be observed. An example of the relation between number of spikes and stimulus concentration is given in Fig. 5 (unit 29).

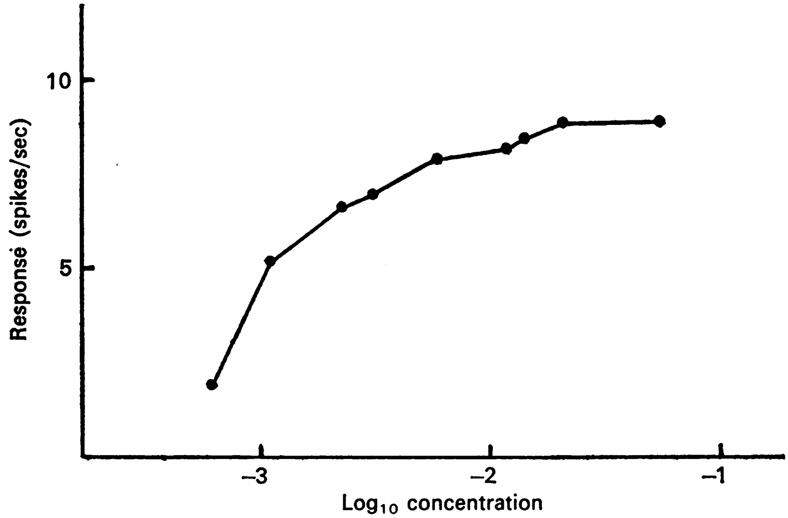

Fig. 4. The relation between stimulus strength (abscissa), and the maximal frequency in the response (ordinate). Stimulant: isoamyl acetate. The experimentally determinable threshold is indicated by *T*. The concentration units represent $\log_{10}$ of the saturated vapour pressure at 20 °C.

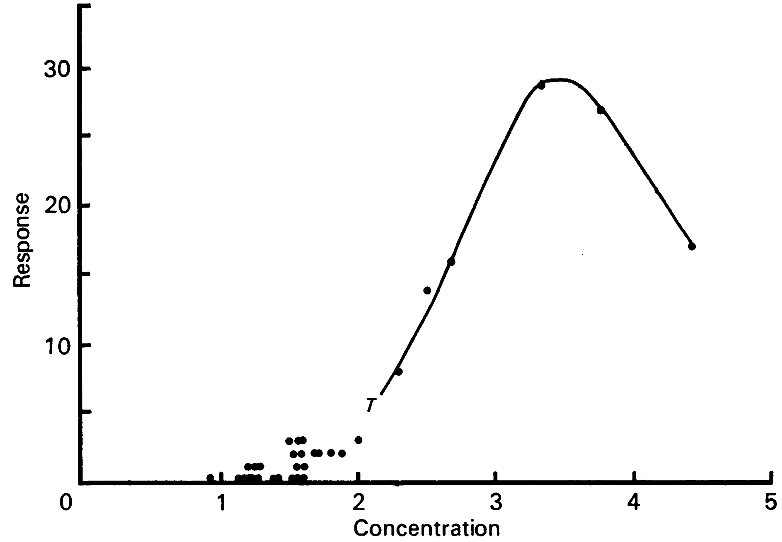

Fig. 5. Diagram of a recorded stimulus–response relation. Unit 29. Stimulant: cineole. The number of spikes counted in the 22 sec after the onset of stimulation plotted against the stimulus concentration. Units on the abscissa represent 1/3200 of the saturated cineole vapour pressure at 20 °C. Note that at high concentrations the number of spikes diminished. Square regression analysis showed an almost linear rise of the response just above the threshold.

Summated activity

Class 1. To visualize the responses to sub-threshold stimulations of the same unit, the neural activity obtained in a number of individual odour trials can be superimposed. An example of the procedure is given in Fig. 6. In this Figure twenty-one sub-threshold odour trials with isoamyl acetate have been arranged according to the concentration as measured by flame ionization. A clear response could be observed in trial 6, no response was displayed in trial 4. In the other trials no decision could be made. When the spike activities (during 54 sec) of the individual odour trials are superimposed, the bottom trace is obtained. It can be seen that there is an increased activity following the onset of N_2 flow, and a conspicuous response to odour stimulation.

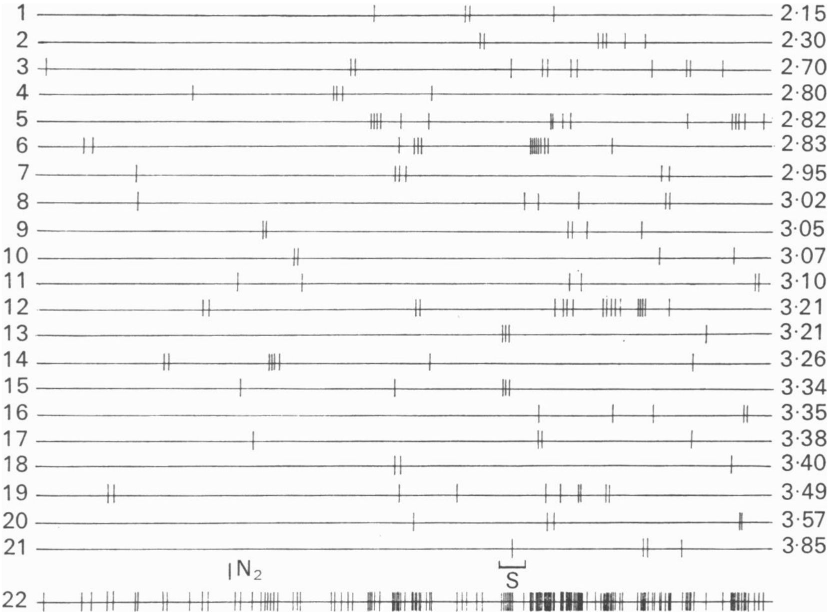

Fig. 6. Unit 24. Stimulant: isoamyl acetate. A number of measurements with stimulus concentrations below the experimental determined threshold are shown (traces 1–21). The concentrations as measured by the flame ionization detector are indicated for each trace. The concentration units indicate a fraction 1/6092 of the saturated isoamyl acetate vapour pressure. A super-position of traces 1–21 is shown in trace 22. Stimulation is indicated with the horizontal bar.

It was not always possible to give a large number of sub-threshold odour trials. In the cases in which only a few odour trials were given, a visible response in one of the individual odour trials (as in trace 6, Fig. 6) was not always observed. For example, in unit 28 no response was observed in six individual odour trials. Nevertheless, a superimposition of the activity of these odour trials showed a clearly visible rise of spike occurrence density 3–7 sec after onset of olfactory stimulation.

Class 2. The high spontaneous activity of the class 2 units obstructed a manual superimposition as shown in Fig. 6. For this reason a somewhat different procedure has been used. For each odour trial the sequence of 54 sec was divided in 2 sec intervals, and the number of spikes occurring in each interval was counted. The counts of the corresponding intervals for several odour trials were added, and plotted against the interval number (time). Two examples of the results of this procedure are given in Fig. 7. In the graphs an augmentation of the activity during N_2 flow can be seen. In unit 17 (Fig. 7*A*) a gradual rise of the summated activity during N_2 flow is shown. A retarded N_2 response or a random fluctuation in the summated activity is present in the 15th sample interval of unit 5 (Fig. 7*B*). If the units responded, an augmentation of the summated activity could be observed after the stimulation (unit 17, Fig. 7*A*). No such clear rise is visible in Fig. 7*B*, although a Wilcoxon test gave a significant difference ($P < 1\%$), in the activities during 20 sec before and after stimulation for each odour trial.

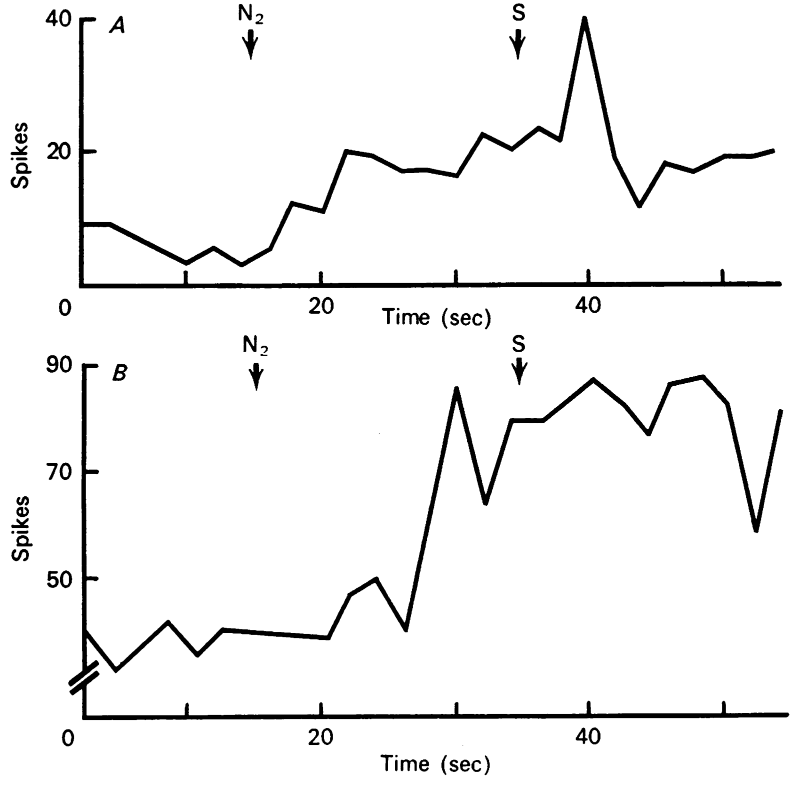

Fig. 7. The summated activity plotted against time (sample interval number). The onset of the N_2 flow and stimulation are indicated by N_2 and S respectively. *A*, unit 17, stimulant isoamyl acetate; stimulus concentrations for the individual odour trials (four) were 1/3385 of a saturated isoamyl acetate vapour pressure. *B*, unit 5, stimulant butanol; stimulus concentrations for the individual odour trials (fifteen) were 1/328 of a saturated butanol vapour pressure.

An augmentation of neural activity during N_2 flow was observed in all cases in which summated activity was considered. This might be due to a mechanical effect of the weak flow. However, it is more probable that the N_2 flow transported the room pollution to the mucosa. Because of the low concentration of this pollution, a response was not visible in the individual odour trial recordings but became visible when considering summated activity.

Statistical analysis

An odour puff delivered at the mucosa results in the absorption of a fraction of molecules in the mucous layer. After having been absorbed, the odorant molecules diffuse through the mucus and each of them may have a successful encounter with one or several olfactory receptor cells. These events carry a number of incertitudes in respect to the reproducibility of the experimental stimulus–response relation. For instance, the flame ionization signals showed that the stimulus concentration in air fluctuated between the odour trials. The number of molecules absorbed by the mucous layer will fluctuate as a function of temperature and of

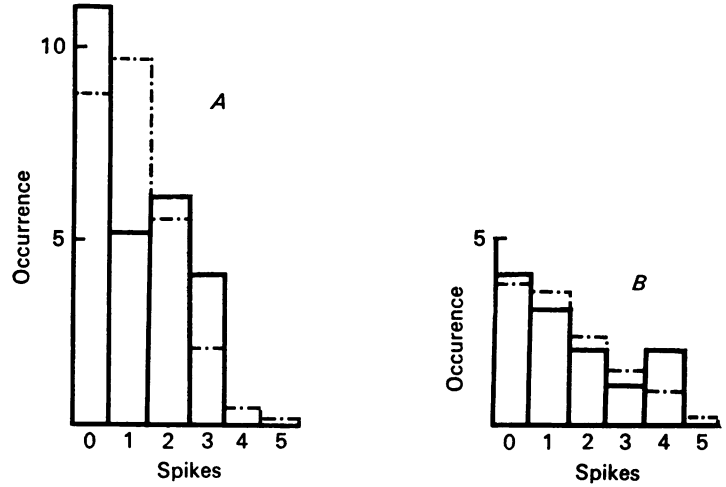

Fig. 8. Histograms of a bad and good fit for the applied theoretical distributions. Recorded distributions are indicated with the heavy line, the theoretical values with the dotted line. *A*, a bad fit (unit 29) was found for a Poisson distribution. *B*, the best fit (unit 27) was found for a modified Poisson distribution. The corresponding significance levels can be found in Table 1.

TABLE 1. Goodness of fit of a Poisson distribution and of a modified Poisson distribution for the number of spikes in the responses (seven units). The percentage indicating the probability of real difference existing based on a chi-square test are represented together with the degrees of freedom and calculated chi-square values

Unit and odorant	Poisson distribution (chi-square significance level)		Modified Poisson distribution (chi-square significance level)		Degrees of freedom
11 Cineole	1·14	50–75%	0·29	25–50%	1
19 Isoamyl acetate	1·36	75–90%	0·04	10–25%	1
21 Cineole	0·23	10%	0·31	10–25%	2
22 Cineole	0·42	10–25%	0·39	10–25%	2
24 Isoamyl acetate	7·71	75–90%	1·00	10–25%	5
27 Isoamyl acetate	0·03	10–25%	0·01	5%	1
29 Cineole	3·42	75–90%	2·56	50–75%	2

local odorant concentration in air, whilst the molecules in the mucus may diffuse in all directions. So one can predict only approximately what response will follow a stimulation at a certain concentration. For this reason statistical distributions have been applied in order to quantify the response.

Spike number distribution. As is shown above, a fluctuation in the response of the receptors may be expected when the concentration of odorant is near a threshold value (Fig. 5). For each of the units with low spontaneous activity (class 1) a response histogram was constructed. Two examples of such histograms are shown in Fig. 8, where frequency of occurrence is plotted for each number of spikes per interval of 22 sec. From the observed figures, the expected values based on a Poisson distribution were calculated (Fig. 8*A*, dotted line). The goodness of fit between the observed and theoretical values was calculated by the chi-square method, combining classes 2 and 3 (chi-square values for frequencies of occurrence under 5 are not reliable). The chi-square value was found to be 3·42, that is to say the recorded values are described by the Poisson distribution as sufficient, i.e. a fit to a Poisson distribution can not be rejected. Fig. 8*B* shows a similar treatment of twelve odour trials with isoamyl acetate to unit 27. Observed values are indicated by the heavy line, theoretical values based on a modified Poisson distribution by the dotted line. The modified Poisson distribution gave a chi-square value of 0·01, indicating that the distribution of the number of spikes occurring in the responses was significantly described by the theoretical distribution.

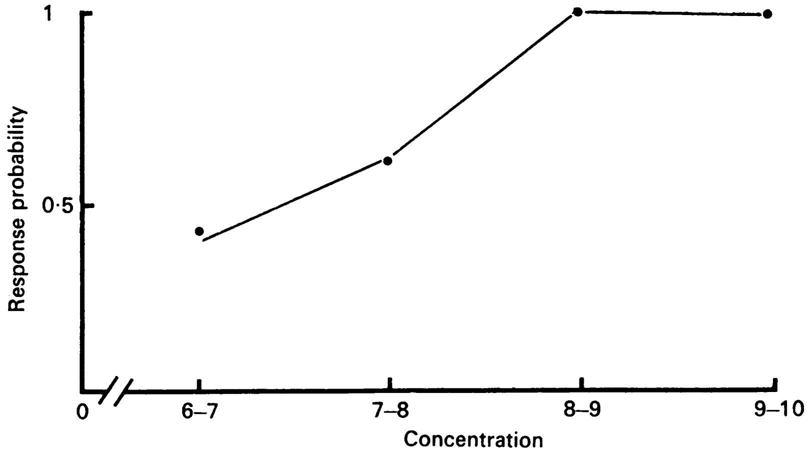

Fig. 9. The relation between the recorded response probability plotted against different concentration classes. Unit 19. Stimulant: isoamyl acetate. The line represents a theoretical curve based on a modified Poisson distribution. The concentration units indicate a fraction of 1/2046 of the saturated isoamyl acetate vapour pressure.

For seven units, the distributions of spike occurrence in individual odour trials were modelled with the Poisson distributions. The calculated chi-square values for goodness of fit, with the corresponding significance levels (indicating the probability of real difference existing), and the degrees of freedom are shown in Table 1. In all cases, with the exception of unit 21, goodness of fit was better with the modified distribution.

Response probability and concentration. It seems reasonable to assume that the probability for eliciting a response increases with stimulus concentration. Whether an odour trial will be successful or not is dependent upon the concentration of the odorant not only above but also below the so-called threshold value. To test this hypothesis, the number of responses occurring after several stimuli within a small concentration range was determined. Only silent units were used, as simple recognition of a response is possible exclusively in units that do not discharge spontaneously. The spike number distribution measured in the same concentration range was used to calculate the probability of obtaining no spike (= no response). Both Poisson distributions were used in the theoretical calculations. When this calculated probability was subtracted from 1, a theoretical value for the probability of a response was obtained. An example of the procedure is shown in Fig. 9. The percentage of responses occurring in several odour trials is plotted against the related concentration class. From this Figure it can be seen that the response probability rises with stimulus concentration. The theoretical values for the response probability based on a modified Poisson distribution are indicated in the Figure (line). The chi-square values for goodness of fit of the exact and modified Poisson distributions indicated significance levels of 80 % and 2·5 % respectively. From this it may be concluded that the response probabilities for different concentration classes are described as significant by a modified Poisson distribution.

DISCUSSION

Neural convergence

A large neural convergence ratio has been demonstrated in a number of vertebrate species. For example in the rabbit about 25,000 olfactory fibres become available for about twenty-five secondary cells in the olfactory bulb (Allison & Warwick, 1949). This means that the signals of about 1000 receptor cells are impinging on the membrane of a secondary cell. An idea of what happens when weak receptor cell activity is amplified can be obtained by comparing the superimposed activity shown in Fig. 6 with the activity displayed in the individual odour trials. This indicates that the so-called near-threshold responses of the receptor cells might be important for information processing in the bulb. A comparison of determined receptor cell thresholds and behavioural thresholds of *Bombyx* (Kaissling & Priesner, 1970) has revealed that the former is 10–100 times higher than the latter. Such a difference in thresholds can be accounted for by an olfactory system with convergence. A recent study by Müller & Kiepenhever (1976) mentions a reaction of the frog to low concentrations of an odorant. However, a comparison of the thresholds using the same odorant is necessary to verify an eventual difference between receptor cell thresholds and behaviour thresholds. The present study gives evidence that the sub-threshold responses of olfactory receptors are essential for odour detection by the olfactory system.

Receptor cell sensitivity

Below threshold concentrations, units displayed a random post-stimulus activity. Confirmation of this statement is obtained by regarding the individual odour trials shown in Fig. 6. Initially it appears that the unit 'responded' independently from stimulus concentration, indicating an insensitive stimulus–response relation. Nevertheless a representation of the response probability (Fig. 9) shows that the number of responses observed after several stimulations is heavily dependent upon the applied stimulus concentration. Furthermore, a better fit between the modified Poisson distribution and the spike number distribution, as compared with the non-modified model has been found (Table 1). This finding might be explained if for each stimulus concentration a response is drawn from a Poisson distribution characterized by a certain expectation (mean value), which in its turn depends upon the stimulus strength even within small concentration ranges. A theoretical example of such a mechanism is represented in Fig. 10. Here three stimulus concentrations ($c_1 < c_2 < c_3$) with the corresponding probabilities to elicit no response, or to respond with 1, 2, 3, etc. spikes, are represented. It can be seen that the response probability, i.e. 1 minus the probability to give no response, depends upon stimulus concentration. The probability that the receptor cell responds with 1, 2, 3, etc. spikes is proportional to the applied stimulus concentration. This indicates that in a statistical fashion a sensitive stimulus–response relation characterizes the olfactory receptor cells.

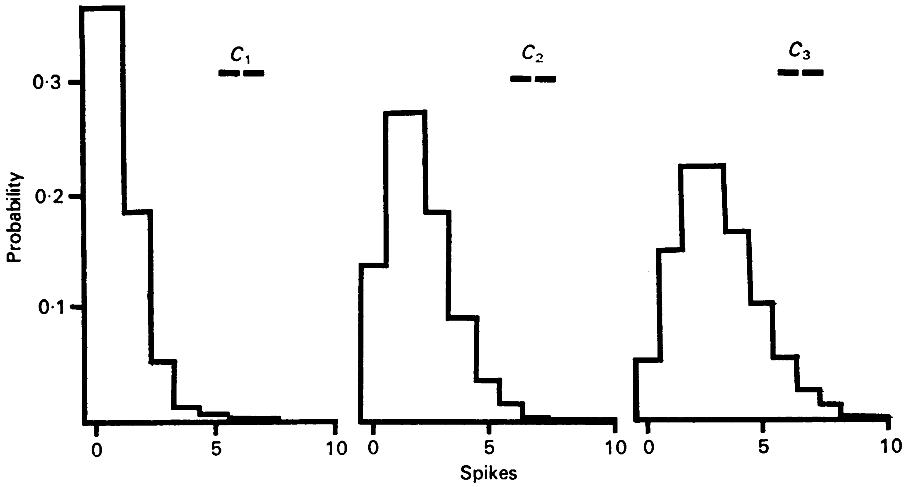

Fig. 10. Three different stimulus concentrations, each with a graph of the corresponding probability to encounter 0, 1, 2...10 spikes. The response probabilities are 0·64, 0·87 and 0·95 for the concentrations c_1, c_2 and c_3 respectively.

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REFERENCES

ALLISON, A. C. & WARWICK, R. T. T. (1949). Quantitative observations on the olfactory system of the rabbit. *Brain* **72**, 186–197.

BOSTOCK, H. & POYNDER, T. H. (1972). Apparatus for delivering and monitoring a sequence of odour stimuli. *J. Physiol.* **224**, 14–15.

COX, D. R. (1962). *Renewal Theory*, Science paperbacks. London: Methuen.

VAN DRONGELEN, W. (1978). Responses of frog olfactory receptors to stimuli at threshold concentrations. Abstr. in *Olfaction and Taste* **6**, Information Retrieval Ltd., London.

VAN DRONGELEN, W., HOLLEY, A. & DØVING, K. B. (1978). Convergence in the olfactory system: quantitative aspects of odour sensitivity, *J. theor. Biol.* **70** (in the Press).

DUCHAMP, A., REVIAL, M. F., HOLLEY, A. & MACLEOD, P. (1974). Odor discrimination by frog olfactory receptors. *Chem. Senses and Flavor* **1**, 213–233.

GESTELAND, R. C. (1976). Receptor membrane functions in olfaction: implication from single unit studies. *Structure–activity relationships in Chemoreception*, pp. 161–168. Information Retrieval Ltd., London.

GESTELAND, R. C., HOWLAND, B., LETTVIN, J. Y. & PITTS, W. H. (1959). Comments on microelectrodes. *Proc. Instn Radio Engrs* **47**, 1856–1862.

GETCHELL, T. V. (1974). Unitary responses in frog olfactory epithelium to sterically related molecules at low concentrations. *J. gen. Physiol.* **64**, 241–261.

HOLLEY, A., DELALEU, J. C., REVIAL, M. F. & JUGE, A. (1976). Quantitative stimulation of frog olfactory receptors: concentration–response relationship. 2nd Congress of E.C.R.O., Reading, U.K.

HOLLEY, A. & DØVING, K. B. (1978). Receptor sensitivity, acceptor distribution, convergence and neural coding in the olfactory system. In *Olfaction and Taste* **6**, Information Retrieval Ltd., London.

KAISSLING, K. E. & PRIESNER, E. (1970). Die Riechswelle des Seiden-spinners. *Naturwissenschaften* **57**, Heft 1, S. 23–28.

MORONEY, M. J. (1956). *Facts from Figures*, Penguin Books, Harmondsworth, G.B.

MÜLLER, W. & KIEPENHEVER, J. (1976). Olfactory orientation in frogs. *Naturwissenschaften* **63**, Heft 1, S. 49–50.

O'CONNEL, R. J. & MOZELL, M. (1969). Quantitative stimulation of frog olfactory receptors. *J. Neurophysiol.* **32**, 51–63.

POYNDER, T. H. (1974). Response of the frog olfactory system to controlled odour stimuli. *J. Soc. cosmetic Chem.* **25**, 183–202.

REVIAL, M. F., DUCHAMP, A. & HOLLEY, A. (1978). Odour discrimination by frog olfactory receptors: a second study. *Chem. Senses and Flavor* **3** (in the Press).