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ANALYSIS OF THE ONSET PHASE OF OLFACTORY BULB UNIT RESPONSES TO ODOUR PULSES IN THE SALAMANDER

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SUMMARY

1. A method for delivering odour pulses of controlled onset, steady plateau and abrupt termination, has been developed and applied to a single unit study of mitral cell responses in the olfactory bulb of the salamander. The pulses have been monitored during the experiments near the site of stimulation on the olfactory mucosa.

2. Responses have been categorized as excitatory or suppressive based on the initial response to the odour pulse.

3. Initially excitatory responses had sustained discharges near threshold. With increasing concentration, the discharge changed to a brief burst followed by suppression. The briefest latency of a unit response was 120 msec, using stimulation of medium concentration, after the start of the pulse; the majority of units appeared to be excited within 200–300 msec. Ramp stimuli gave increasing periods of excitation as the rise time of the odour front became less abrupt.

4. Initially suppressive responses showed suppression at all levels of concentration. The majority of units appeared to have an onset of suppression about 300–400 msec after the start of the pulse.

5. These basic responses, involving suppression or excitatory–suppressive sequences, can be correlated with some basic properties of the synaptic circuits in the olfactory bulb. The time courses of the initial responses appear to be within the time periods of the inhalation cycle of the salamander, and therefore may reflect mechanisms of processing of natural olfactory stimuli.

INTRODUCTION

We report here the results of a study in the salamander, in which odour stimuli have been delivered in the form of step pulses and ramps with defined onset phases, steady plateaus and rapid terminations. The pulses

have been monitored, and the single unit responses of mitral cells in the olfactory bulb have been correlated with their time courses. This type of stimulus control is common in the analysis of other sensory systems (e.g. retina (Kuffler, 1953); muscle receptors (Ottoson & Shepherd, 1971)) and we believe it can also be an effective method for olfactory studies.

A previous study (Kauer, 1974) has suggested that the initial phase of sensory input to the olfactory bulb is crucial in setting up the basic response patterns of bulbar cells to given odours at given concentrations. The emphasis of the present study is on the basic properties of this earliest phase of the mitral cell responses. The results give support to the functional importance of a differentiation between excitatory and suppressive response types, as previously reported (Kauer, 1974; Kauer & Moulton, 1974; Kauer & Shepherd, 1975*a*), during the onset of odour-induced activity in the olfactory bulb. We shall discuss how these response properties may be related to the synaptic circuits in the bulb, and to mechanisms of odour processing.

METHODS

Animal preparation and recording system

Salamanders (*Ambystoma tigrinum*) of both sexes were immobilized, mechanically held and locally anaesthetized as previously described. The surgical exposures and recording techniques have been previously indicated (Kauer, 1974). Extracellular unit recordings were made with 4 M-NaCl-filled micropipettes. In each experiment simultaneous recording of bulbar spikes, electro-olfactogram (EOG) and odour monitor were made.

Immobilization by pithing vs. curare. To control for possible effects of tubocurarine on the CNS, some animals were immobilized by pithing the neuraxis anteriorly and posteriorly. No discernible differences in the temporal patterns, sensitivity, or responsiveness of the bulbar units to the various odorants were seen using each of these methods.

While there were no differential effects on response patterns, pithing was not as satisfactory as curare for several reasons. First, it was necessary to pith anteriorly in order to reduce reflexive respiratory movements. This procedure often caused increases in blood pressure as seen by the flow in the vessels on the surface of the bulb. Secondly, pithed animals tended to show more copious mucus secretion into the nasal cavity than curarized animals, so much so that in some cases the cavity would become filled and the experiment had to be discarded. Thirdly, units in pithed animals were more unstable in terms of being able to record from them for long periods of time. This may have been due to circulatory pulsations related to the increase in blood pressure.

Recording site verification. Electrical stimulation to elicit bulbar orthodromic and antidromic evoked potentials was carried out through concentric bipolar stimulating electrodes. Changes in these potentials were used to locate the unit recording sites. The electrodes were made of insulated silver wire threaded into stainless-steel hypodermic needles with their ends ground flat. The orthodromic electrode was placed on the cranial side of the bundle of incoming olfactory nerves (ON) and the antidromic electrode on the posterior mediodorsal aspect of the olfactory bulb, the site of the medial olfactory tract (MOT). The positions of the stimulating electrodes,

recording electrodes and odour delivery nozzle are shown schematically in Fig. 1. Electric shocks were monophasic, centre electrode negative, constant current pulses which lasted from 10 to 500 μsec, depending on the experiment.

During the initial penetration in search of spontaneously active single units, the reversal of the field potential evoked with either ON or MOT stimulation was used as a guide for the layer of mitral/tufted cell bodies. In addition the bulbar micropipettes were filled with 4 M-NaCl saturated with fast green (Thomas & Wilson, 1965) for dye-marking the recording site at the end of the experiment.

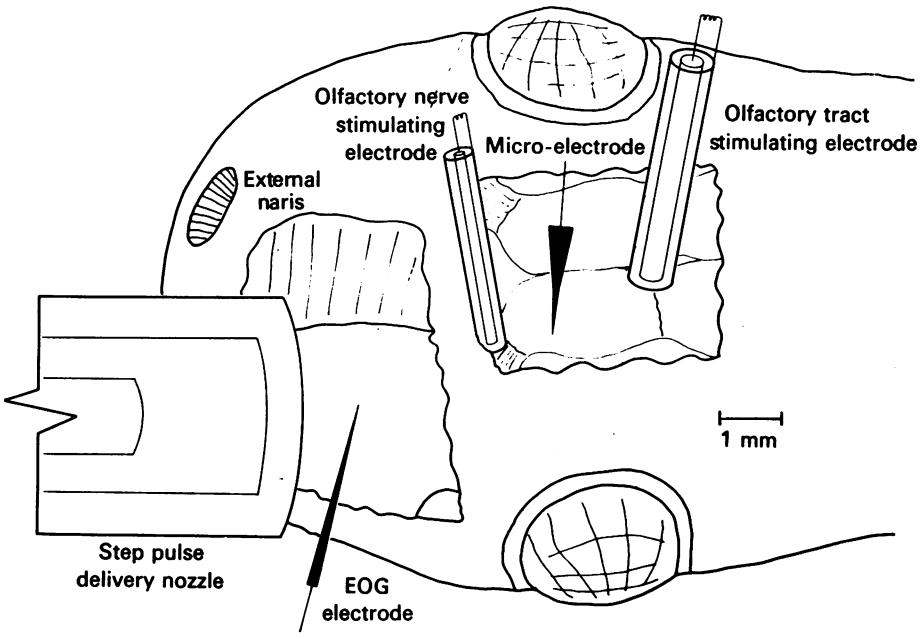

Fig. 1. Schematic diagram of experimental set-up showing positions of odour delivery nozzle, EOG and spike recording electrodes, and olfactory nerve and medial olfactory tract stimulating electrodes.

Odour control and stimulation methods

Olfactometer. Control of the concentration and flow rate of the odours was achieved using the olfactometer previously described (Kauer, 1974). The major components of this system are shown in Fig. 2. This system has been calibrated with regard to concentration using a gas–liquid chromatograph (Kauer, 1974). In the present experiments the carrier gas was 95 % O_2, 5 % CO_2.

Concentrations of the odour stimuli were calculated using the ideal gas law and are expressed as molarity of the stimulus compound in the vapour stream coming from the delivery nozzle. In this way direct comparisons between concentrations among the odours may be made. It should be noted that for odours with very different vapour pressures, equal concentrations may never be reached in the animal's natural environment. Thus comparisons made between such odours delivered at equal molarities may give misleading results in terms of the way in which the system performs in the natural state.

Nozzle and monitor. The time course of stimulation was controlled and monitored 'on-line' by the system previously described by Kauer & Shepherd (1975). The delivery nozzle and inlet to the analyser which monitored the CO_2 in the carrier are shown in Fig. 2*B*, *D*. The carrier flow rate was 136 ml./min through a nozzle with a final diameter of 2 mm. The flow rate of the control vacuum through the centre nozzle was 400 ml./min; the surround vacuum was 100 ml./min. The vacuum flow rate on the inlet to the Beckman CO_2 analyser was 400 ml./min.

In some of the Figures variations or bumps on the monitor trace are seen. These are due to slight fluctuations in the ambient air during delivery of the pulse or to contamination by CO_2 in the breath of the investigator (see Fig. 10*D*).

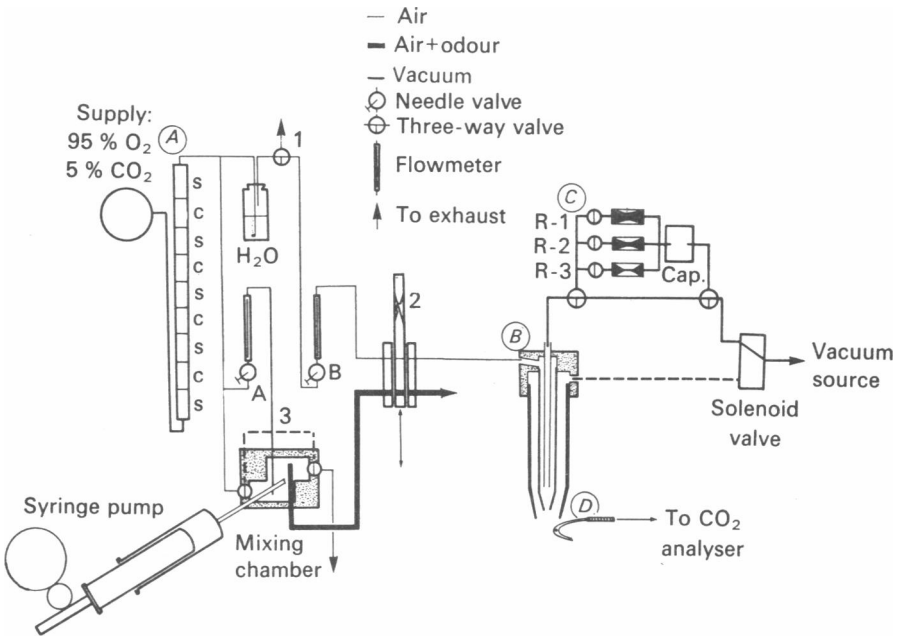

Fig. 2. Diagram of odour delivery system including olfactometer (*A*), odour delivery nozzle (*B*), ramp generator (*C*) and CO_2 pulse monitor (*D*). s, silica gel; c, activated charcoal; 1, pressure release valve; 2, slide valve to direct either pure carrier stream or odour plus carrier to delivery nozzle; 3, ganged valves to admit wash air to mixing chamber; A, valve and flowmeter controlling carrier stream to mixing chamber; B, valve and flowmeter controlling pure carrier flow to delivery nozzle; R-1, 2, 3, various lengths of small-bore Teflon tubing serving as resistors in ramp generator; Cap., 250 ml. Erlenmeyer flask serving as capacitor in ramp generator.

Rise time control and calibration. In the present system the rise time of the pulse was determined by the speed with which the control vacuum (in the centre nozzle) could be shut off. Under optimal conditions, measurements using the Beckman CO_2 analyser indicated that the rise times of the 'square' pulses ejected from the nozzle were on the order of 80–100 msec to reach 70% of peak amplitude. Since this is within the rise time of the Beckman analyser itself, it is possible that the pulse was rising somewhat faster. We have not made any more precise determinations of these

delays. On the slow time scale of the salamander responses, the onset of the pulse was sufficiently abrupt for the purpose of our analysis of spike patterns. A pulse with a rise time of approximately 100 msec was apparently rising more rapidly than the salamander olfactory system could follow, as reflected by the EOG and spike responses.

Ramps. Fig. 2*C* illustrates that part of the delivery system which was used to generate odour pulses having leading edges with different rise times. This system operated on the centre vacuum line of the nozzle which controls the onset of the odour; if this vacuum stream was abruptly switched off, the odour was abruptly switched on. In this way 'square' pulses were generated. Similarly if the vacuum was gradually turned off, the odour was gradually turned on. The rate of decay of this vacuum stream could be readily controlled by inserting a resistance and capacitance in series in the line. In analogy with electrical circuits, this changes the RC time constant of the system. In the present case the 'resistors' were made of lengths of small-bore Teflon tubing and the 'capacitor' was an Erlenmeyer flask. This system used a 250 ml. flask as the capacitor and various lengths (6, 12 and 30 cm) of AWG no. 18 Teflon tubing as the resistors.

The rising phases of the ramps were calibrated using the Beckman monitor. Since the presence of the Beckman vacuum distorted the shape of the ramps, these pulses were calibrated off-line and the Beckman could not be used for monitoring the ramps during the experiments. The shapes of the ramps, with their rise times to 90% of the plateau, are shown in Fig. 8. The rise times were manipulated over the range of 0·2–3·4 sec by connecting different resistances into the line (see Fig. 2*C*).

Odour delivery-procedure. Odours were introduced into the olfactometer as previously described (Kauer, 1974) and allowed to equilibrate with the tubing and valves for 1–2 min. The odours used for eliciting responses were chosen from among the group described in a previous study (cf. Kauer, 1974). Those most commonly used were isoamyl acetate, cineole, camphor, pinene and menthol. We wish to emphasize that our main concern in this study has been to characterize physiological rather than odour discrimination mechanisms *per se* and for this purpose the selection of odours on the basis of molecular structure has not been a critical consideration. To deliver a series of pulses the stimulus delivery valve (Fig. 2, valve no. 2) was switched so that the odour stream was directed to the three-barrelled nozzle. It may be noted that in the present system there was no on-going clean carrier playing continuously onto the nasal mucosa (cf. Kauer, 1974), but the carrier plus odour was switched on by the nozzle only during the presentation of a stimulus. Blank pulses (carrier alone) for one and two seconds, made before odour applications for each unit, were delivered by adjusting the stimulus delivery valve so that the clean carrier stream went to the nozzle and the odorized carrier was directed to the exhaust.

Although the abrupt front of carrier stream constituted a potential mechanical stimulus to the olfactory receptors, we observed a response to the carrier stream alone in only one unit. This response could have been due either to mechanical stimulation or to some trace contaminant in the carrier stream or simply to the carrier stream itself. The fact that the present stimulation method gave a response using non-odorized pulses in only one case and that the response types seen in this study are fully compatible with those observed using a system specifically designed to prevent the delivery of a mechanical pulse with odour presentation (Kauer, 1974) has given us confidence that mechanical effects are not a significant contributing factor to the responses we observed.

While the odorized air was running through the nozzle, stimulus applications were made at a rate of not more than one per minute. The onset and duration of the pulses were determined by an electrical stimulator controlling the solenoid valve.

Changes in concentration were made by changing the speed of the syringe pump controlling the flow of saturated odour vapour. The olfactometer and nozzle were washed out between odours by running the clean carrier stream through them.

RESULTS

Unit localization

We have obtained stable recordings from 111 olfactory bulb units. The recording sites of all units were localized by noting the wave pattern of the associated evoked potentials. In general pattern these potentials were similar to those evoked by olfactory nerve and lateral olfactory tract volleys in other species (e.g. rabbit: Phillips, Powell & Shepherd, 1963).

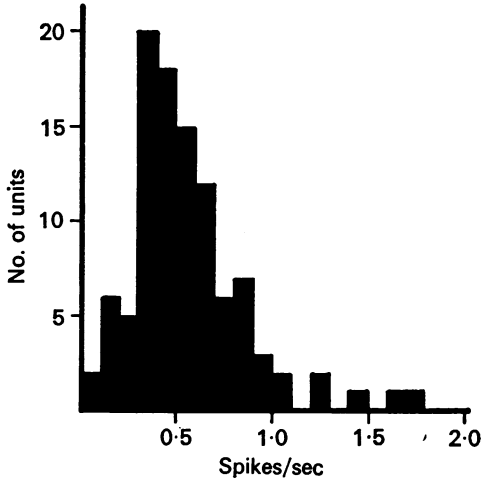

Fig. 3. Histogram of average spontaneous activity of units found in mitral/tufted cell body layer of salamander olfactory bulb. Rates were measured for approximately two minutes prior to odour application.

All of the units were at or near the depth at which occurs a transition between negative and positive potentials during period III of the response (Rall & Shepherd, 1968). This has been correlated with the layer of mitral cell bodies (Phillips *et al.* 1963), and we conclude that most, if not all, the recordings were therefore from mitral cells. In addition, in twenty-three experiments the unit recording sites were dye-marked, and these were all within the mitral body layer. An example is shown below in Fig. 9.

Spontaneous activity

For each of the units the spontaneous spike activity was recorded over a 2 min period before the start of odour stimulation. Fig. 3 shows a histogram of the average spike frequencies for this period for the entire unit

population. The peak can be seen to lie at 0·3 spikes/sec, or approximately 20 spikes/min. This agrees well with the value of 0·5 spikes/sec reported previously (Kauer, 1974), and is also similar to the value of 0·7 spikes/sec reported in frog olfactory bulb (Døving, 1964). A relatively slow rate of spontaneous spike activity thus appears to be characteristic for the olfactory bulbs of these species. The patterns of discharge were irregular, and showed variation in different units, as can be seen in the following Figures. We did not observe regular trains or repetitive bursts. Spontaneous firing rates above 1–2 spikes/sec rarely lasted more than a brief period, and were taken to reflect injury and have not been included in our analysis.

Excitatory responses

Responses which begin with an increase in the rate of spike discharge have been classified as excitatory (*E*). As in the original report (Kauer, 1974) we have found that these responses include several patterns of excitation, and excitation followed by suppression, and that these patterns are clearly dependent on the intensity of odour stimulation.

A typical example is shown in Fig. 4, for a unit which responded to the odour of amyl acetate. Just above threshold (*A*) the response consisted of a sustained discharge lasting through most of the period of stimulation. The discharge appears to be more or less continuous with the preceding spontaneous activity, but close comparison with the stimulus monitor allows the onset of the response to be determined (arrow). Repeated runs gave assurance, in this and other experiments, that the onset of the response at a given odour intensity was reproducible (cf. Kauer, 1974). The responses to pulses of increasing concentration (decreasing molar dilution) are shown in *B–E*. The shortening in duration of the initial discharge is evident. At the higher intensities (*C–E*) the initial excitation is followed by a suppression which begins during the period of stimulation. There was also suppression for several seconds in the aftermath of the stimulus; this period changed relatively little with stimulus intensity. The spontaneous activity returned at approximately the same frequency as before stimulation in all the trials.

These excitatory responses are shown on faster sweeps in Fig. 5*A*. In *B* are shown instantaneous frequency plots for these responses. It can be seen that the initial latency decreased from 450 to 120 msec; the duration of the discharge decreased from 1200 to 100 msec; and the peak frequency increased from 8 to 12·5 impulses/sec, as the odour concentration was raised. The relation between response frequency and duration varied in different units, indicating that the two properties are to some extent independent. The termination of the discharge also marked the onset of

suppression, so that one could also conclude that the onset of suppression shortened in latency as the concentration of the stimulus increased.

The short latency and very brief duration were the two chief and striking characteristics of the initial excitatory responses at medium to high odour

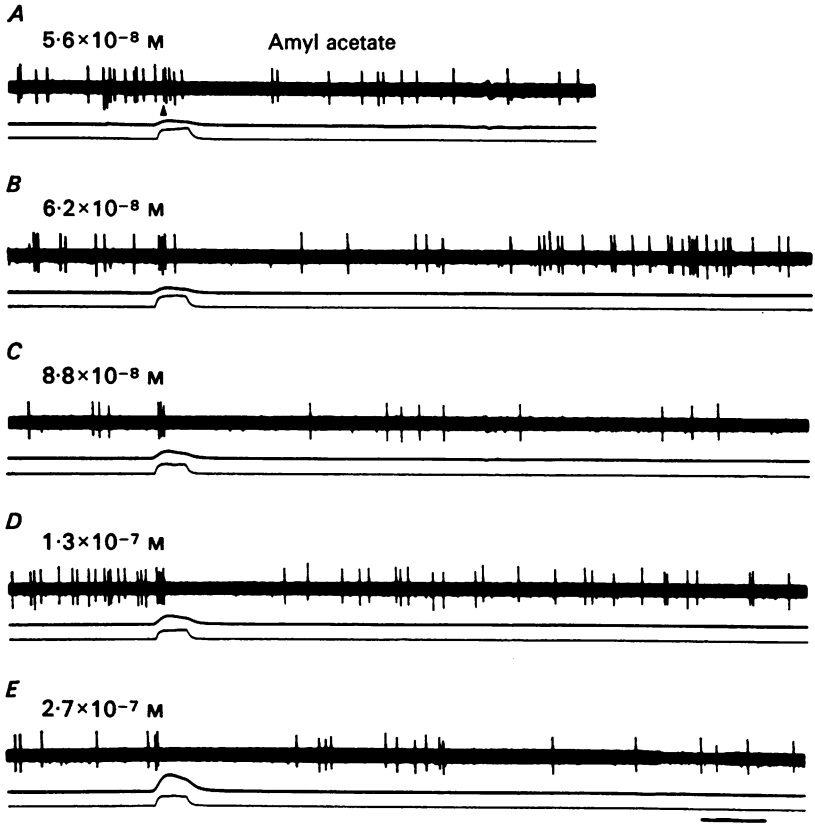

Fig. 4. Excitatory responses of a bulbar unit to 2 sec pulses of increasing concentrations of amyl acetate in an animal immobilized with curare. Top trace in each record is spike recording from olfactory bulb; second trace is EOG from olfactory epithelium; third trace is output of odour monitor. Just above threshold (*A*) the response consists of an excitation lasting for the duration of the pulse followed by a period of suppression after the odour has been turned off. As the concentration is raised (*B–E*) the duration of the burst is curtailed, the latency of the onset of the response is shortened and the onset of the suppression begins earlier. Time calibration = 4 sec. Spikes have been retouched.

concentrations. Examples for different units and different odours are shown in Fig. 6. The values for the initial latency, the intervals between spikes, the discharge duration, and duration of the suppression, are given in the table in the Figure legend. The brevity of the excitatory discharges, and

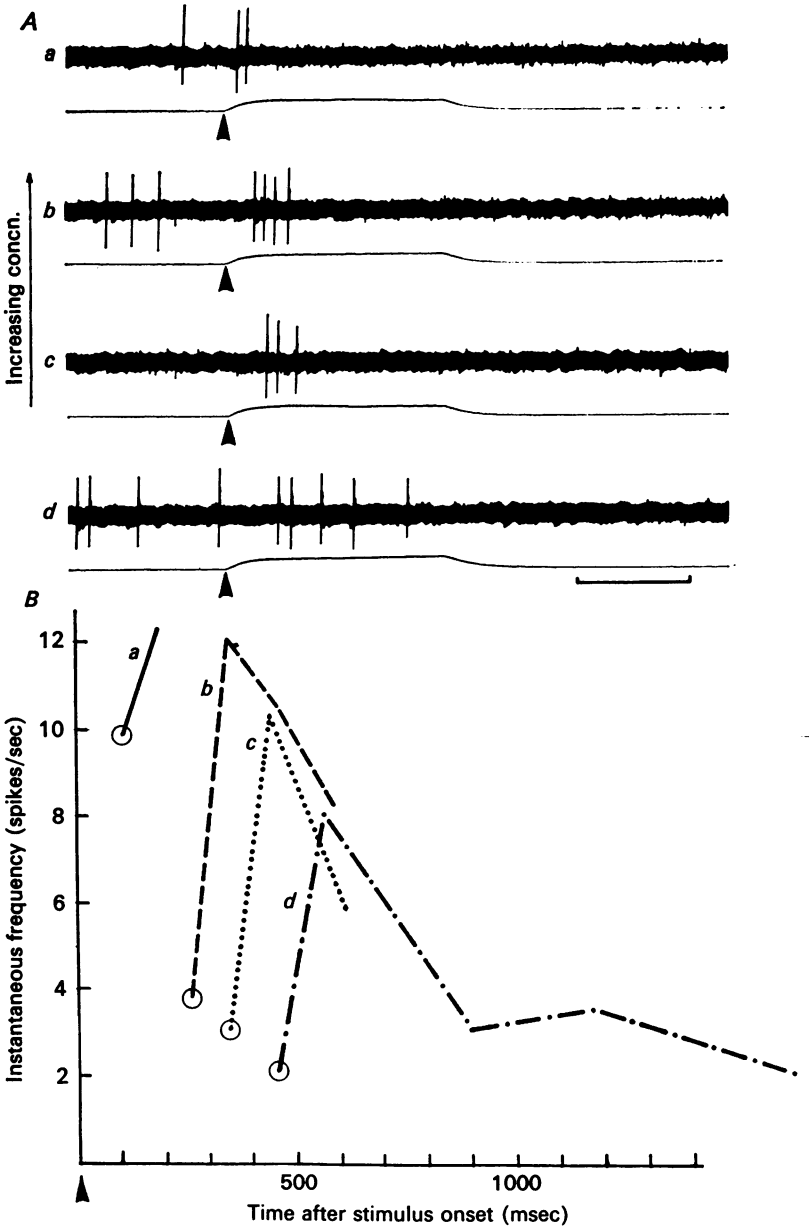

Fig. 5. *A*, fast sweep records of traces from Fig. 4, showing detailed time course of mitral layer unit to decreasing concentrations (*a–d*) of amyl acetate. Time mark = 1 sec. Spikes have been retouched. *B*, graph showing instantaneous frequency of spikes in each of the records above. Circled points indicate latency after onset of stimulus. Arrows in all records and graph indicate stimulus onset.

the relatively long duration of the ensuing suppression, are notable. The values for these four units are representative for the entire population of excitatory responses in our series. It is important to note that the responses were not specific for either units or odours; they were obtained in different units and with different odours, as is indicated in Fig. 6. As is also shown in the Figure, they were obtained in both curarized and pithed animals.

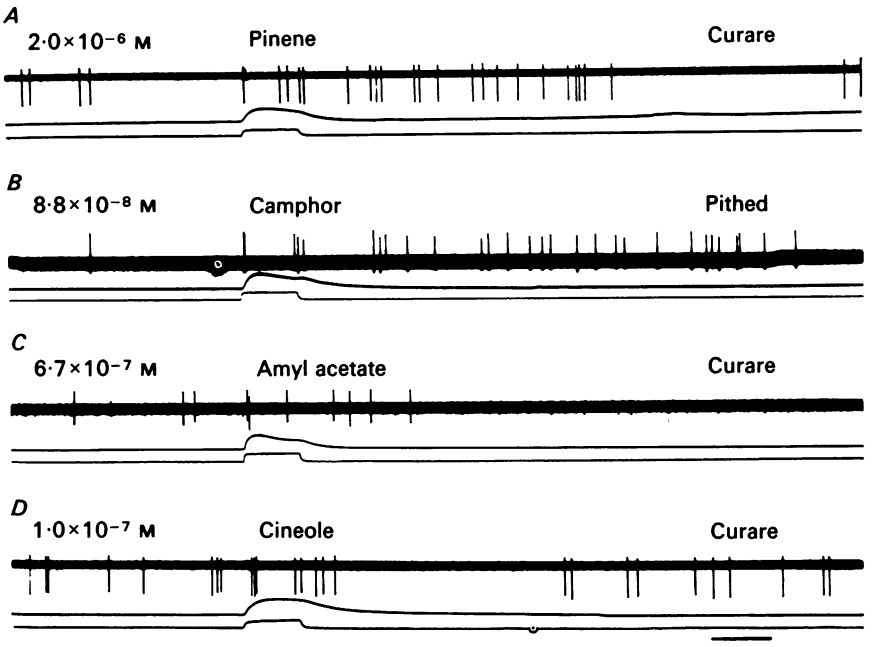

Fig. 6. Four different units showing initial excitation, followed by suppression, followed by excitation (response category E_3; Kauer, 1974) possibly followed by subsequent suppression to 4 sec pulses of four different odours. *A*, *C* and *D* are from animals immobilized with curare. *B* is from an animal which has been pithed. Units *B* and *C* were identified as being in the mitral/tufted body layer by dye-marking. Time calibration = 4 sec. Spikes have been retouched

Unit	Onset latency (sec)	Spike interval	Discharge duration	Suppression duration (sec)
A	0·3	1 spike	1 spike	2·46
B	0·24	0·1 sec	0·2 sec	3·50
C	0·03	0·12 sec	0·14 sec	2·65
D	0·55	0·24, 0·1 sec	0·34 sec	2·72

We would like to emphasize (see also Methods) that these responses were obtained at relatively low odour concentrations. The threshold concentrations for the excitatory discharges lay generally in the range of

10^{-6} to 10^{-9} M concentration. The brief early discharges usually emerged (as in Fig. 4) within 1–2 log steps of the threshold concentration. We have never observed complete suppression of an early excitatory discharge at higher stimulus intensities, but this point was not thoroughly investigated because of our desire to avoid the unphysiological effects of high odour concentrations on the olfactory epithelium.

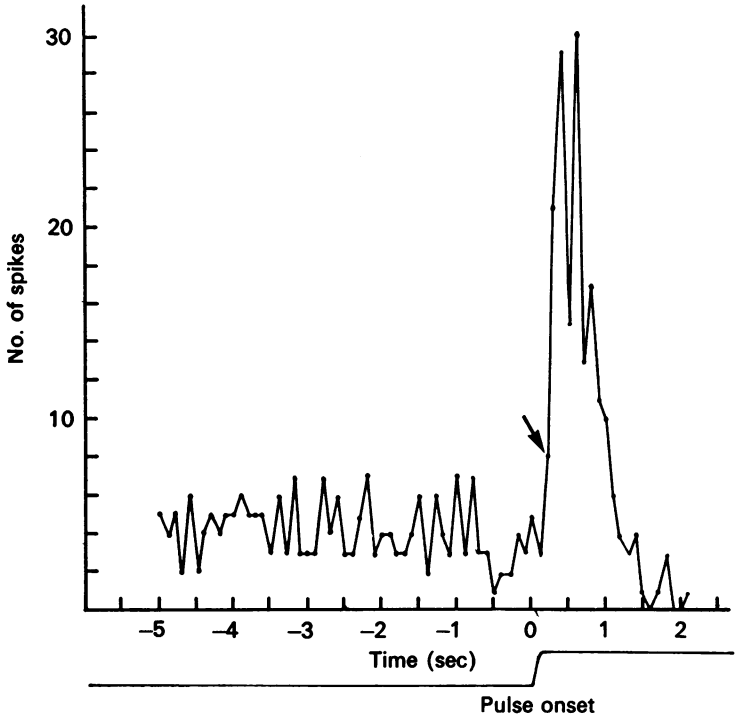

Fig. 7. Histogram of eighty-two applications of different odours to different units which showed excitatory temporal patterns similar to those seen in Fig. 6. The numbers of spikes in each 100 msec bin have been plotted for 5 sec preceding and 2 sec following the onset of the pulse. Notice that the point at 200 msec (arrow) after the pulse falls above the maximum during pre-stimulus time. This indicates the first onset of excitation in this population. Note the dip in the peak of excitation at 500 msec.

Figs. 4 and 6 show that the onset of an excitatory discharge could usually be determined unambiguously even in single trials despite ongoing background activity because of the precise timing of the first spike relative to the onset of the monitored pulse. This of course was clearest at higher stimulus intensities. The timing of the excitatory activity is of importance for understanding the neural mechanisms for odour processing in the olfactory bulb (see Discussion), and we have therefore sought further

evidence on this point by pooling the recordings from a number of units showing excitatory responses. Fig. 7 shows a histogram for the time of occurrence of spikes in the 5 sec period before a pulse and the first 2 sec after its onset; the ordinate is simply the total number of spikes in 100 msec time bins for the total of eighty-two trials. The first point on the curve above the highest level of spontaneous discharge occurs at the interval of 200–300 msec after the onset of the pulse. This appears to set

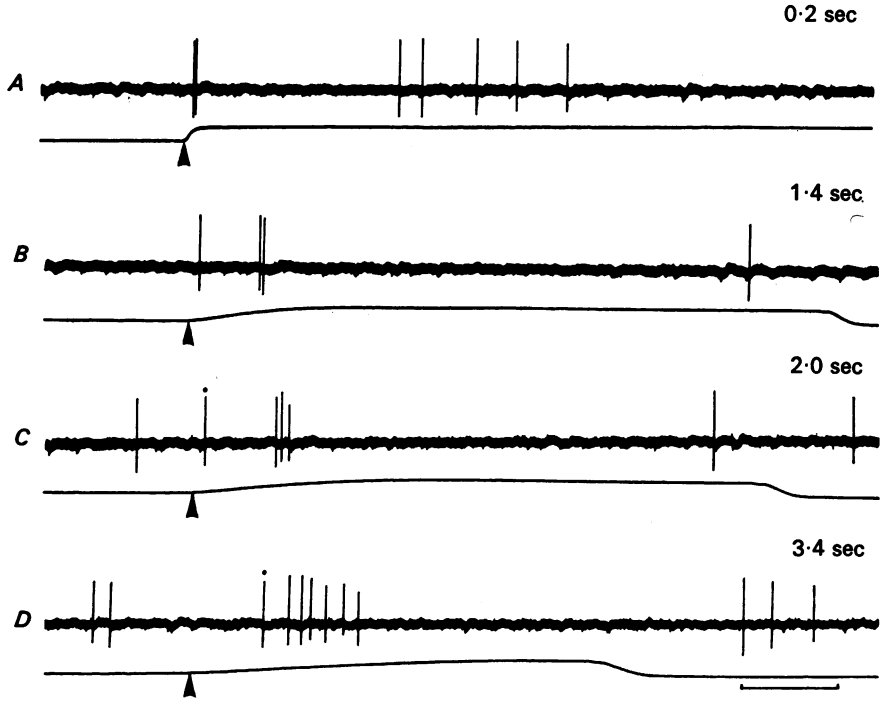

Fig. 8. Unit responses to cineole delivered as a square pulse (*A*) and as pulses with leading edges having different rise times (ramps *B–D*). Each response consists of an initial excitation followed by suppression during the pulse. As the rising phase of the odour onset becomes progressively slower (*B–D*) the duration of the initial burst becomes longer and the latency of the first spike in the burst increases. Arrows mark the onset of the pulses. Spikes with a dot above are part of spontaneous activity. Numbers to the right indicate the rise time of the pulses. Time mark = 1 sec. Spikes have been retouched.

a lower limit for the latency of onset of excitation in the bulb with these pulse stimuli. Note the prominence of the peaks at 400 and 600 msec; this reflects the intensity of the discharges (short intervals between spikes) and the fixed timing of the excitatory process in different runs. The sharp

decline to near zero at 1·5–2·0 sec presumably reflects the fixed timing of onset of suppression, and its relatively powerful nature.

Ramp stimuli

With the pulse stimuli, the responses changed characteristically as odour concentration was raised (Fig. 4). It appeared that the rate of rise of the stimulus was in fact more rapid at higher concentrations, as judged by the decrease in latency and burst duration and increase in frequency. The relation between the prolonged excitatory discharge and the concentration range over which it can be elicited is a critical one, and we wished to examine it in more detail. This was studied by the use of slowly rising ramps of odour (see Methods). The unit responses in Fig. 8 were recorded for four different rates of rise, all to the same final concentration plateau. As the stimulus rise became faster, the response duration decreased, latency decreased, and frequency increased. On a slow time scale these changes are strikingly similar to those seen with the rapid onset pulses in Fig. 4. A reasonable interpretation is that at the slowest rate of rise, the cell fired over a specific range of concentration, and as the rate of rise increased and the time spent during that concentration range shortened, the duration shortened correspondingly. Because of the uncertainties noted in the Methods regarding the delays in the odour monitoring system, a precise determination of the odour range, and its similarity at different rates of rise, was not possible.

Suppressive responses

In the original study (Kauer, 1974) suppressive (S) responses were identified as those which showed only suppression during the early phase of stimulation; they were thus differentiated from the E responses in which excitation is followed by suppression, as described in the preceding section. An example is shown in Fig. 9. This unit was localized in the mitral cell body layer (photomicrograph above). In *A–C* are shown trials with the odour of amyl acetate at three concentrations, spanning approximately a two log concentration range. We have already discussed the question of identifying a response against a background of ongoing activity, and the same points apply with these responses (see also below). Spike activity was absent during each of the 4 sec pulses, and for variable periods (1–8 sec) afterward (the spike at the onset of the pulse in *B* is part of the spontaneous activity; see Fig. 11 below). We follow the previous study in terming as suppression the absence of activity. The suppression is complete at threshold, and there is no change with increasing concentration.

These results illustrate a salient feature of the exclusively suppressive (S) responses: they show little of the sensitivity to concentration that is

shown by the excitatory-suppressive sequences of E responses. The suppression in the aftermath of a pulse could also be independent of odour concentration, as in Fig. 9. In this unit the spontaneous activity returned to approximately its level before stimulation, for all stimulus intensities.

The S responses were not specific for either particular units or odours; they were obtained in different units and with different odours, as is shown

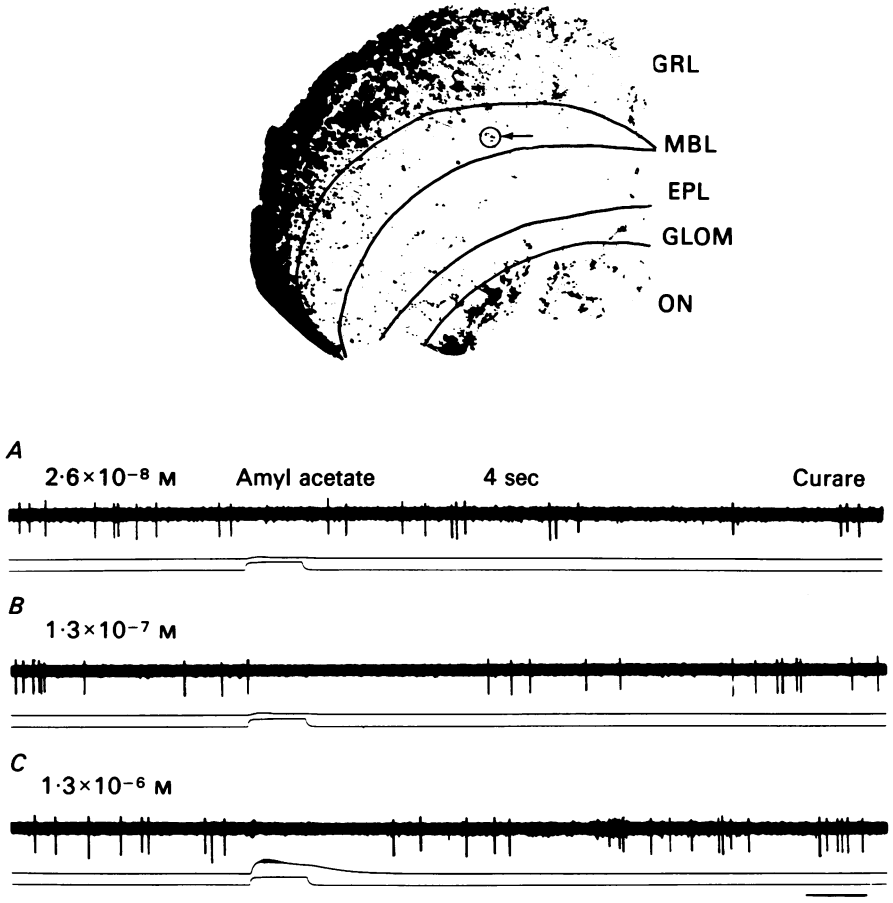

Fig. 9. Top: photograph of cross section of left olfactory bulb showing dye mark for unit shown below. Lines on photo show layers of the bulb: GRL, granule cell layer; MBL, mitral/tufted body layer; EPL, external plexiform layer; GLOM, glomerular layer; ON, olfactory nerve layer. Dorsal is at the top, lateral to the right. Bottom: records *A–C* are from the unit whose position is shown above. This cell gave suppressive responses to 4 sec pulses of amyl acetate at three log steps of concentration. This animal was immobilized with curare. Time calibration = 4 sec. Spikes have been retouched.

by the examples in Fig. 10. They were obtained in both curarized and pithed animals, as also indicated in the Figure. Note the relatively low molar stimulus concentration. This gives confidence that the S response does not reflect unphysiologically high concentrations of odour stimulation of the olfactory receptors. Thus, apart from the lack of initial excitation and the apparent lack of dependence on odour concentration, the S responses share certain basic properties with the E responses.

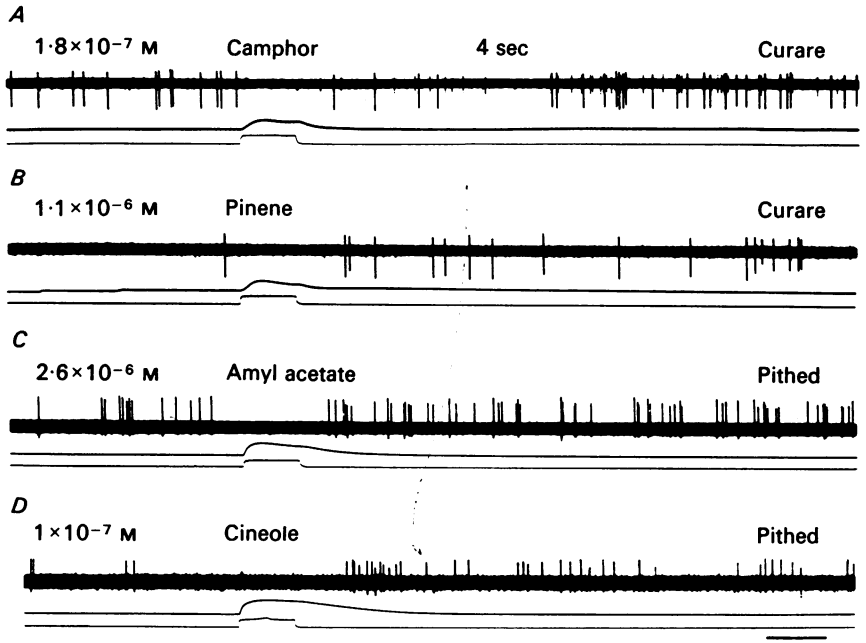

Fig. 10. Four different units showing uniform suppression (response category S_1; Kauer, 1974) to 4 sec pulses of four different odours. *A* and *B* are from animals immobilized with curare, *C* and *D* are from animals that have been pithed. Units *B–D* were identified as being in mitral/tufted body layer by dye-marking. Time calibration = 4 sec. Spikes have been retouched.

The lower the rate of spontaneous activity the more difficult it is to identify a purely suppressive response; in these cases, repeated runs are necessary. Fig. 11 illustrates such an experiment; a representative trial is shown at the bottom, and above is a histogram of the total number of spikes in 1 sec bins summed over six trials, for the period of 15 sec before the pulse and 30 sec after its onset. The pulses, of camphor vapour, lasted 4 sec. It is clear that the only time intervals without any spikes occur during the odour pulse.

We wished to determine as accurately as possible the time of onset of

suppression, and this clearly could not be done in single trials, or even in repeated trials with one odour and one unit. We have therefore followed the procedure used in Fig. 7, and pooled the data from 127 trials in fifteen units, and the results are shown in Fig. 12. The first point below the level of spontaneous activity occurs at the interval of 300–400 msec after the onset of the pulse, and the suppression is complete by about 0·5–0·7 sec. In comparison with the results in Fig. 7, this indicates that when only suppression occurs, it begins slightly later than the excitation of the E responses (latency 200–300 msec) but is complete at an earlier time than the suppression in the E response (1·5–2 sec). In both cases the ensuing suppression is complete (no spikes occur despite the large numbers of runs).

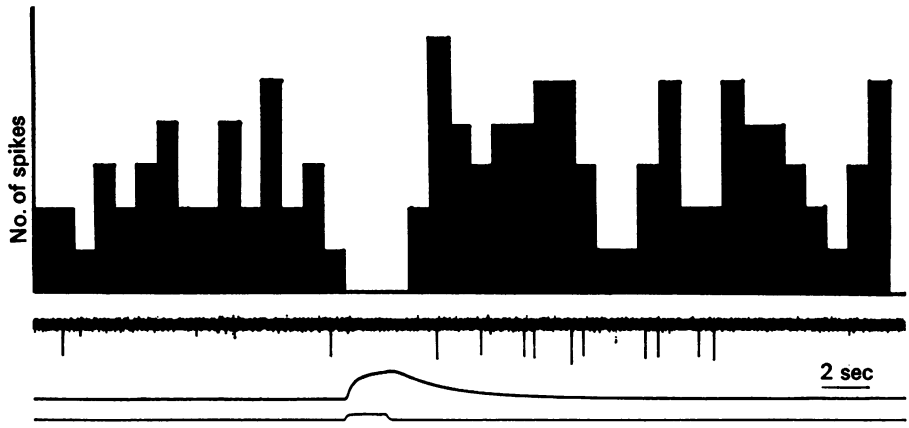

Fig. 11. Histogram of six runs from the same unit as shown below, stimulated under exactly the same conditions. The number of spikes per 1 sec bin have been summed from the six runs. The absence of spikes during the 2 sec odour application and for 1 sec following stimulation is clear. Record of unit with slow spontaneous activity showing suppression during 2 sec odour pulse of $4{\cdot}4 \times 10^{-8}$ M camphor. Suppression is not obvious when compared to long silent periods in absence of stimulation. Spikes have been retouched. Time calibration = 2 sec.

DISCUSSION

Importance of monitored pulses

The precise wave form of the natural odour stimulus within the nose during inhalation is not known for the salamander, or for any species. In the absence of such information, the step pulse has seemed a logical wave form with which to begin. It has a simple, defined time course, which is accepted as a basic tool in the analysis of other sensory systems. This type of stimulus has not been used previously in unit studies of the olfactory system, apart from our preliminary reports (Kauer & Shepherd, 1975*a*, *b*).

A second problem in studying a sensory system is to monitor the stimulus. The standard practice in olfaction has been to use the EOG for this purpose. Although the EOG is closely dependent on the stimulus, it is in fact a recording of the summed response of the olfactory receptors (cf. Ottoson, 1971). It is a valuable, indeed necessary, monitor of overall receptor responsiveness, but not of the stimulus *per se*. For this reason we have felt it essential to have an independent record of the stimulus itself.

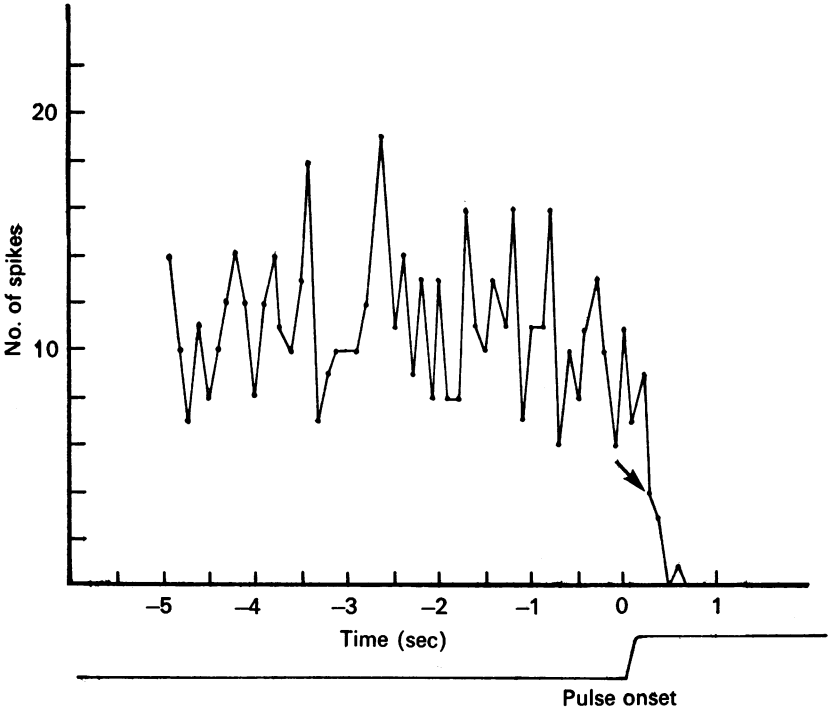

Fig. 12. Histogram of 127 applications of different odours to different units which showed suppression. The numbers of spikes in each 100 msec bin have been plotted for 5 sec preceding and 1 sec following the onset of the pulse. Note that the point at 300 msec (arrow) after the pulse falls below the minimum during pre-stimulus time. This indicates the first onset of suppression in this population.

The utility of a step pulse of odour, monitored near the site of stimulation, is apparent we believe in the present experiments. Stimulus duration and concentration could be independently varied. The initial abrupt onset was clearly distinct from the steady plateau of the stimulus. The termination of the pulse was not only abrupt, but also involved active removal of odorous molecules from the space above the olfactory epithelium. This not only meant that changes in unit activity were relatively independent of

the presence of odour molecules in the aftermath of a pulse, but it also reduced contamination between pulses to a minimum and helped preserve the viability and responsiveness of the epithelium. The responses appeared to be very reproducible in successive trials. By reference to the stimulus monitor, the unit response could usually be clearly identified, whereas, without the monitor, it would have been difficult to do this, particularly in single trials. Thus, the monitored pulse technique has the added practical advantage of reducing the number of trials necessary to establish a particular response pattern.

Properties of excitatory responses

The most striking patterns of activity induced by the odour pulses were the responses which began with excitation. Our results confirm the original description of these responses (Kauer, 1974) and support the proposal that they constitute a specific response type.

An important property of this type is the dependence of its response pattern on odour intensity. Thus, responses which have a pattern of slow prolonged discharge are not an entirely separate category from responses which have a pattern of excitation followed by suppression; the one grades into the other as odour intensity is changed. The property of a cell to give a sustained discharge over a given range of odour concentration has been termed 'concentration tuning' (Kauer, 1974). The results from the use of ramp stimuli support the inferences drawn from the pulse stimuli with regard to the importance of this property. In the terminology of sensory processing, the threshold response can be regarded as slowly adapting, whereas the brief excitation at high intensities can be regarded as extremely rapidly adapting.

With regard to the site and mechanism of the E type discharge in the bulbar neurones, we have only indirect evidence. The threshold response would appear to reflect excitatory input from the receptors, perhaps driven by relatively asynchronous receptor activity. At higher stimulus intensities one may assume that the rapid onset of the odour pulse causes a relatively synchronous activation of some part of the receptor population, and that this is in turn transmitted as a barrage of impulses in the olfactory nerve. The short latency and high frequency of the initial spike discharge in the bulbar neurones presumably reflects the earliest arrival of this input. The brief duration of the discharge could reflect the briefness of the input volley or some self-limiting property of the bulbar cells, but it must also be due in large part to the intervention of the process of suppression which shuts off further spike activity during the plateau phase of the odour pulse.

The anatomical arrangement of the olfactory bulb, in which the olfactory nerves all terminate within the olfactory glomeruli, means that one

can pinpoint the site of the initial excitatory synaptic input there. The suppression, on the other hand, could have several sites. The sequence of excitation followed by suppression is similar to the sequence of synaptic excitation followed by suppression that has been demonstrated in bulbar neurones in the rabbit following a single electrical shock to the olfactory nerves (Shepherd, 1963; Getchell & Shepherd, 1975). There is evidence that the suppression of mitral cells is largely due to a recurrent dendrodendritic pathway for synaptic inhibition through granule cells (Phillips *et al.* 1963; Rall, Shepherd, Reese & Brightman, 1966; Rall & Shepherd, 1968; Nicoll, 1969). The action of this pathway is long-lasting, and could readily account for the long-lasting suppression observed in the present experiments. An additional possible site for inhibition is in the glomeruli, by means of dendrodendritic and axodendritic pathways through the periglomerular (PG) cells (Pinching & Powell, 1971; White, 1972, 1973; Shepherd, 1971; Getchell & Shepherd, 1975). These studies of synaptic circuits have been carried out mainly in the mammal, but there is considerable evidence that the basic synaptic connections and physiological properties are similar in many vertebrate species (cf. Andres, 1970; Shepherd, 1972).

A possibility that must also be considered is that the response properties in bulbar units could reflect in part the response patterns of receptor cells in the periphery. Consideration of this point is hampered by the fact that previously reported receptor cell responses have not been elicited by monitored pulse stimuli. A feature of most of these studies is a wide variety of response patterns, rather than the limited and stereotyped categories we see (cf. Gesteland, Lettvin, Pitts & Rojas, 1963; Gesteland, Lettvin & Pitts, 1965; O'Connell & Mozell, 1969; Holley, Duchamp, Rivial, Juge & MacLeod, 1974; Getchell, 1973, 1974). Many of the excitatory responses begin with a long latency and greatly outlast the stimulus pulse. This also has not been observed in our study. Responses in which there is decrement in spike amplitude and inactivation of the spike response have been reported in receptors, but not seen in our study. A few examples of units which respond with bursts of decreasing duration to increasing concentration have been seen (Gesteland *et al.* 1963; Shibuya & Tucker, 1967; Matthews, 1972; Holley *et al.* 1974). However, none of these have the same time course as in the bulbar responses.

A study of olfactory receptor unit responses in the salamander, using a similar apparatus for delivering step pulses and for pulse monitoring, has shown that olfactory receptors respond with stereotyped patterns, and that these differ in certain definite respects from the responses in the bulb (T. V. Getchell & G. M. Shepherd, in preparation).

The evidence thus suggests that the excitatory–suppressive sequences

at intermediate odour concentrations and above are mainly established by bulbar circuits mediating synaptic excitation in the glomeruli followed by synaptic inhibition through either the granule cells or PG cells, or both.

We cannot of course rule out the possibility of more complex processing mechanisms. For example, the initial excitation could be due to disinhibition, from the periphery or at the glomerular level, and the suppression could be due to disfacilitation. However, it seems more fruitful to pursue possible mechanisms in terms of the considerable anatomical and physiological evidence already available.

Properties of suppressive responses

The significance of suppression for the generation of E responses has been discussed above, and it remains to discuss the purely S response. We have no evidence for its site or mechanism, beyond the inhibitory circuits through granule cells and periglomerular cells already mentioned above. An additional possibility is through longer circuits passing into the forebrain and back to the olfactory bulb through centrifugal fibres (Kerr & Hagbarth, 1955; Callens & Boisacq-Schepens, 1963; Døving & Gemne, 1966; Price, 1968, 1969; Broadwell & Jacobowitz, 1976). The purely S response may involve these or other circuits that are different from, or activated in different sequences and to differing extents than, the suppression that follows the initial excitation of E responses. Evidence for this has been obtained in preliminary experiments using pulses of long duration (Kauer & Shepherd, 1975). It was found that activity returns during the prolonged plateau of the pulse in some E responses but not usually during S responses. Further analysis of the patterns of response during and after long duration pulses will be reported elsewhere (J. S. Kauer & G. M. Shepherd, in preparation).

Functional implications

With the present experimental arrangement, we have delivered odour pulses which have rise times from 100 msec to several seconds. At rest the salamander respires at a rate on the order of 1–2/sec (unpublished observations). The range of onsets of stimulation we have examined therefore includes the time course of onset of a natural inspiration. Thus, it seems likely, to a first approximation, that the faster rates of rise of the pulses are similar to the rates of rise of odour concentration in the nasal cavity during a natural inspiration. This suggests that the initial differentiation into excitatory or suppressive responses seen with the pulses could be part of the mechanism used by the animal in the perception of odour under natural conditions. Our results suggest, in fact, that both odour quality and odour concentration could be discriminated during this brief period.

It is likely, however, that this is only an initial step in odour discrimination, because of the slow time course of the prolonged excitatory discharge at lower concentrations, and the prolonged suppression at all concentrations. This suggests that these may be part of the mechanism used by the animal through several inspiratory cycles to aid in detection and discrimination (cf. Macrides & Chorover, 1972). Experiments with repetitive pulses are in progress to more clearly test this hypothesis.

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