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# SYNAPTIC ACTIONS ON MITRAL AND TUFTED CELLS ELICITED BY OLFACTORY NERVE VOLLEYS IN THE RABBIT

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SUMMARY

1. A unitary study has been carried out of mitral and tufted cell responses to olfactory nerve volleys in the olfactory bulb of rabbits lightly anaesthetized with urethane–chloralose.

2. With volleys of different strengths, some mitral cells responded with a spike whose latency decreased considerably as the strength increased (elastic response); other cells responded at an invariant latency (inelastic response). The former may reflect diffuse olfactory nerve inputs to the dendritic tufts in the olfactory glomeruli, while the latter may reflect input from discrete bundles of fibres.

3. The shortest spike latencies are consistent with monosynaptic excitation by the olfactory nerves; longer latencies may be due to longer pathways through the nerves, or polysynaptic pathways within the glomerular layer.

4. Facilitation, in terms of lower threshold and shorter spike latency, was found when testing with paired volleys of weak intensity at relatively short intervals ($< 40$ msec). Suppression, in terms of raised threshold, longer latency and briefer repetitive discharges, was found at intervals up to several hundred msec. The facilitation and suppression are consistent with the hypothesis of synaptic excitation and inhibition, respectively, mediated through interneurones in the olfactory bulb.

5. Presumed tufted cells were similar in response properties to identified mitral cells.

6. Intracellular recordings revealed long-lasting hyperpolarization and, in some cases, an initial depolarization leading to spike initiation, in response to an olfactory nerve volley.

## INTRODUCTION

The first site for synaptic transmission in the vertebrate olfactory pathway is the glomerular layer of the olfactory bulb. It is analogous in this respect to the outer plexiform layer of the retina (Shepherd, 1970). The glomeruli are spherical regions of neuropil containing the terminals of olfactory axons and the dendrites of projection neurones (mitral and probably tufted cells) and of a species of short-axon cell, the periglomerular cell. These are shown diagrammatically in Fig. 1.

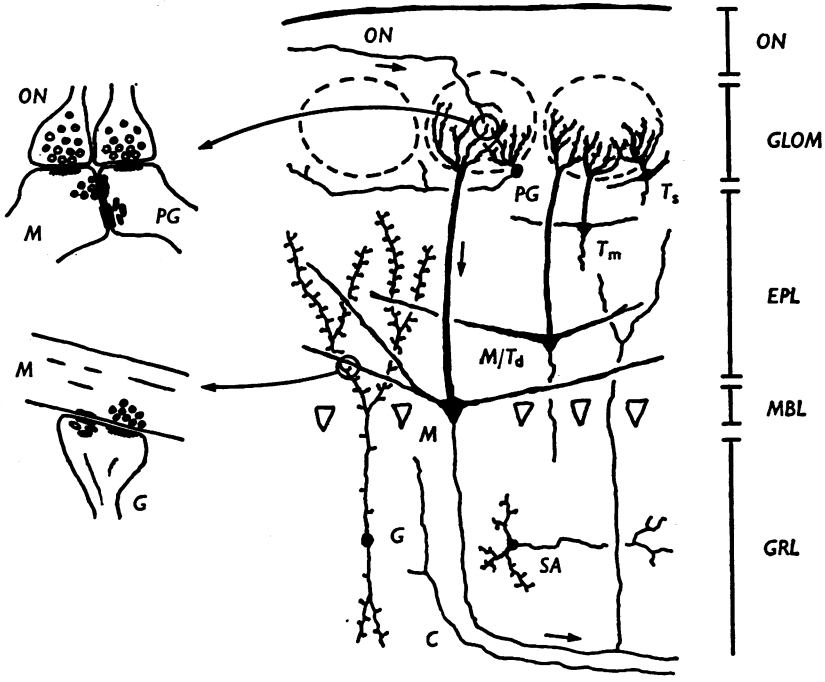


Fig. 1. Schematic diagram summarizing organization of rabbit olfactory bulb: *ON*, olfactory nerves; *PG*, periglomerular short-axon cell; $T_s$, superficial tufted cell; $T_m$, middle tufted cell; $M/T_d$, displaced mitral or deep tufted cell; *M*, mitral cell; *G*, granule cell; *SA*, short-axon cell of deep layer; *C*, centrifugal fibres. Histological layers shown at right: *GLOM*, glomerular layer; *EPL*, external plexiform layer; *MBL*, mitral body layer; *GRL*, granule layer. Insets at left show main types of synaptic connexions in glomeruli (above) and external plexiform layer (below) (see text).

Recent electronmicroscopical studies (Pinching & Powell, 1971*b*; White, 1972, 1973; Willey, 1973) have shown that, in the rabbit, olfactory axons make synaptic contacts on to the dendrites of all three cell types: mitral, tufted and periglomerular cells. In addition, the dendrites of

mitral (and tufted) cells and periglomerular cells are interconnected by numerous dendodendritic synapses, which are seen singly or in serial or reciprocal arrangements (see upper inset, Fig. 1).

Physiological studies in the olfactory bulb have in the main concentrated on the deeper layers, where mitral dendrites have numerous reciprocal synaptic connexions with the dendrites of granule cells, a type of anaxonal interneurone (see lower inset, Fig. 1). It is believed that the granule cells provide a pathway for dendrodendritic recurrent inhibition of mitral cells at that level (Phillips, Powell & Shepherd, 1963; Rall, Shepherd, Reese & Brightman, 1966; Rall & Shepherd, 1968; Nicoll, 1969). It is therefore important to know what interactions take place between the mitral cells and the periglomerular interneurones at the glomerular level, and whether these are mediated by dendrodendritic or axodendritic pathways. The need for this information is enhanced by conflicting reports on the synaptic actions of the periglomerular cells (cf. Freeman, 1974*b*).

Earlier studies showed that mitral as well as periglomerular cells are synaptically excited by a single volley in the olfactory nerves (Yamamoto & Iwama, 1962; Yamamoto, Yamomoto & Iwama, 1963; Shepherd, 1963*a*, *b*). More recently, evidence was obtained for excitatory actions of mitral cell dendrites on to periglomerular dendrites, and inhibitory actions of periglomerular dendrites on to mitral cell dendrites (Shepherd, 1971), which was consistent with inferences from the morphological features of the synapses (see legend, Fig. 1). We here report studies of mitral and tufted cell responses and, in a following paper, of periglomerular cell responses (Getchell & Shepherd, 1975), to single and paired volleys in the olfactory nerves. The results support previous evidence for dendrodendritic synaptic interactions in the glomerular layer of the olfactory bulb.

## METHODS

Experiments were carried out on forty-nine rabbits. Young animals (3–4 months of age; 1·5–3·0 kg body weight) were preferred, because of the high incidence of rhinitis in older animals.

The animals were initially anaesthetized by I.V. injection of a mixture of 10% urethane and 1% alpha-chloralose in mammalian Ringer solution (Phillips *et al.* 1963). An amount, 6–7 c.c./kg. body wt., was given over a period of 1–1½ hr; this long induction period helped to minimize respiratory depression. During surgical procedures halothane (1–2%) in 50% oxygen and air was given. The animal was subsequently maintained at a light level of anaesthesia with either halothane (0·5–1%) or I.P. urethane-chloralose. In several animals maintained on halothane the unitary activity in the olfactory bulb appeared to deteriorate. This could reflect the potentiation of granule cell inhibition reported by Nicoll (1972*b*), though the concentration of halothane was usually much lower than that used by Nicoll. No specific deleterious effects due to the urethane-chloralose were apparent in the experiments, which lasted 12–15 hr. The dosage of chloralose was in any case lower

by almost an order of magnitude than the minimum used by Nicoll to cause potentiation of granule cell inhibition by this drug.

After tracheotomy the left eye was removed to expose the lateral surface of the cranium, and the dorsal cranium was exposed rostrally over the nasal bone. An opening was made over the left olfactory bulb and extended rostrally by careful nibbling with a fine rongeur of the nasal bone at its junction with the cribriform plate. This exposed one or more dorsal bundles of olfactory nerve fibres on their way from the nasal cavity to the olfactory bulb. An opening was also made in the lateral surface of the cranium to expose the lateral olfactory tract on the surface of the prepyriform cortex.

Recordings of unitary activity in the olfactory bulb were made using micropipettes filled with 3 M-KCl or 4 M-NaCl. For extracellular recordings, DC resistances in the range of 10–20 MΩl were optimal. Recordings of giant extracellular spikes, with peak-to-peak amplitudes ranging up to 73 mV, have been previously reported using micropipettes of this type (Phillips *et al.* 1963). Giant spikes have also been obtained in the present study (cf. Fig. 12). It is not known whether the conditions under which such spikes are recorded are associated with some change in the excitability of the neuronal membrane upon which the pipette tip impinges. Our main interest in the present study was in relatively small changes in excitability at threshold levels of synaptic inputs, and we have therefore made no effort to 'grow' spikes to giant size. Instead, units with spikes of relatively small amplitude (1–3 mV), recorded under stable conditions from the time of initial encounter throughout the recording period (10 min to 2 hr) have formed the bulk of the unit population. We assume that under these conditions the pipette tip introduces the least possible change in the normal excitability of the recorded neurone.

A few intracellular penetrations with micropipettes of 20–50 ΩM were obtained (cf. Fig. 12). However, for the reasons stated above, intracellular recordings were not a primary objective of these experiments; a separate study is in progress (S. D. Erulkar & G. M. Shepherd).

Pulsations of the bulb were restrained by a circular disk of X-ray film pressed lightly on to the dorsal surface by a silver wire ring. The micropipette was introduced through small holes in the disk. In some experiments the forebrain was removed by suction to reduce respiratory pulsations.

The olfactory nerves were stimulated through an enamelled silver wire (200 $\mu$m diameter), exposed only at the tip. The long length of the wire (several cm) ensured that the tip rested lightly on one of the exposed olfactory nerve rootlets and maintained a stable relation to the rootlet despite small pulsations of the underlying bulb. This was important during long-term testing of unit excitability using olfactory nerve volleys. Single volleys of impulses were set up in the nerve by shocks of 50 $\mu$sec and longer duration. The shorter durations required larger currents; with pulse durations of 0·5 msec, the thresholds for activation of spike responses in bulbar units ranged from 0·02 to 0·5 mA. We have not systematically compared stimulus currents used in different experiments, and stimulus intensities will therefore be reported here in arbitrary units.

Special care was taken that stimulation did not bring about fatigue of the olfactory nerves, which are composed of non-myelinated axons of 0·2–0·4 $\mu$m diameter. Single or paired volleys were therefore given at a rate of only once every 4 sec. This is similar to the rates of stimulation used by Bliss & Rosenberg (1974) in the tortoise, but slower than the rates (1–2 per sec) used by Freeman (1972, 1974*a*, *b*). Physiological observations on olfactory nerve volleys are reported in the following paper (Getchell & Shepherd, 1975).

The lateral olfactory tract was stimulated through a silver wire which also rested

gently on the surface (Phillips *et al.* 1963). Single shocks set up a volley of impulses in the mitral cell axons of the tract; the impulses spread antidromically into the mitral cell bodies in the olfactory bulb, where, it is believed, they activate a dendro-dendritic synaptic pathway for mitral-to-granule cell excitation followed by granule-to-mitral cell inhibition (Rall *et al.* 1966; Rall & Shepherd, 1968). In some of the present experiments the tract was stimulated in order to assist in identifying the mitral cells by antidromic invasion, and to observe the inhibition of mitral cells over this route. In other experiments the tract was not exposed or stimulated, to avoid introducing any changes in excitability of the bulbar neurones by this artificial procedure.

Two anatomical methods were used to control for sites of unit recordings in the bulb. One was to correlate the recording depth with the summed potentials evoked by an olfactory nerve or lateral olfactory tract volley at that depth, and to relate those depths to a histological section of the bulb containing the recording track, after the method of Phillips *et al.* (1963). The other method consisted of using recording micropipettes filled with fast green dye, and staining the recording site by ejecting dye from the tip according to the method of Thomas & Wilson (1965). The recording depths determined by this method were consistent with the predictions from the observations of the evoked potentials. Further studies to attempt to identify units using intracellular and extracellular staining techniques are in progress.

## RESULTS

### *Unit identification*

Electrode penetrations were made in the same part (anterodorsal) of the bulb in all experiments. The depth of the mitral cell body layer was, with few exceptions, in the range of 600–700 $\mu$m below the surface, as determined by the pattern of antidromic summed potentials and the correlations with histological sections, according to the methods of Phillips *et al.* (1963). This permitted the depths at which units were encountered to be pooled, and the results for 162 units are summarized in Fig. 2.

As can be seen, no units were encountered in the most superficial 100 $\mu$m. This is in agreement with the lack of nerve cell bodies in the surface layer, and further indicates that our pipettes did not record unitary potentials from the olfactory axons there. Similarly, in the depths of the bulb (800 $\mu$m and more), few units were recorded, which is in agreement with various lines of evidence that granule cell bodies, though present in great numbers at these depths, do not generate action potentials (Rall *et al.* 1966; Rall & Shepherd, 1968; Nicoll, 1969; Shepherd, 1972).

Between these levels, the evoked potential patterns and histological correlations indicated that the units recorded at depths of 100–300 $\mu$m fell within the glomerular layer, those from 400–600 $\mu$m within the external plexiform layer, and those from 600 to 700 $\mu$m at the mitral cell body layer (see Fig. 2). The units recorded at the mitral cell body layer could be identified with most certainty; these were mitral cells, unambiguously so when the spikes were of large amplitude and were driven antidromically

from the lateral olfactory tract. Unitary spikes recorded in the external plexiform layer could have two main origins; one is the cell bodies of tufted cells (deep or middle tufted cells), the other is the dendritic trunks of mitral cells. Studies of these units are also reported here. There is increasing evidence that on anatomical (cf. Pinching & Powell, 1971*a*, *b*) and physiological (cf. Nicoll, 1970) grounds the mitral and tufted cells share many properties. Although this is subject to certain qualifications (see Shepherd, 1972), it will be convenient to consider them together for the purposes of this paper.

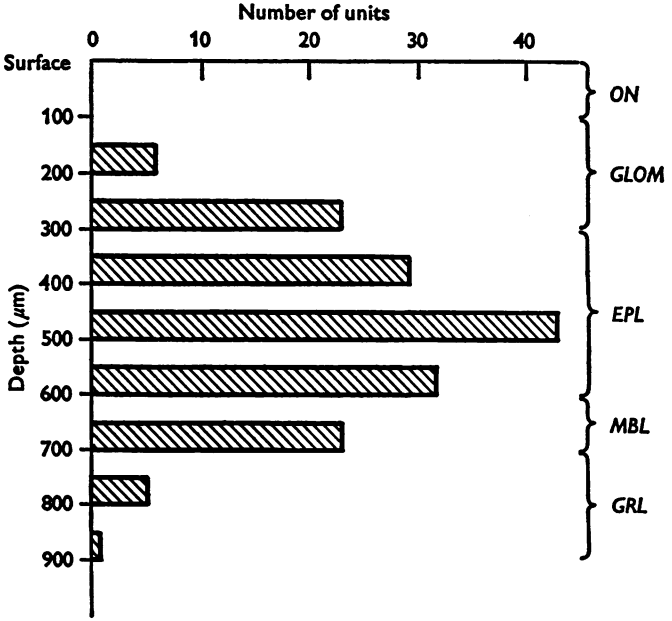


Fig. 2. Depth distribution of 162 units recorded in the olfactory bulb, grouped in 100 $\mu$m increments. Depths indicated on left; estimated correlation with histological layers, on right. The abbreviations used are given in Fig. 1.

Units recorded from the glomerular layer are most probably periglomerular cells, but could also include external tufted cell bodies, mitral and tufted cell dendritic trunks, or extraglomerular short-axon cells. The analysis of glomerular layer units will be considered in a subsequent paper (Getchell & Shepherd, 1975).

*Response properties of mitral units*

The responses to single olfactory nerve volleys of a unit recorded at a depth of 626 $\mu$m are illustrated in Fig. 3. This was identified as a mitral

unit on the basis of its depth and its antidromic response to a lateral olfactory tract volley, as shown in the top trace (*a*). Traces (*b–d*) show the responses to olfactory nerve volleys of different strengths. The threshold response (*d*) consisted of a single spike; at high intensities (*c*, *b*) there was a second spike discharge. The latencies of the spikes relative to the shock artifact are plotted in the graph of Fig. 3*B*. There was a gradual shortening of the latency with increasing shock intensity, but the interval between the spikes was relatively constant, about 4 msec, i.e. the equivalent of about 250 impulses sec.

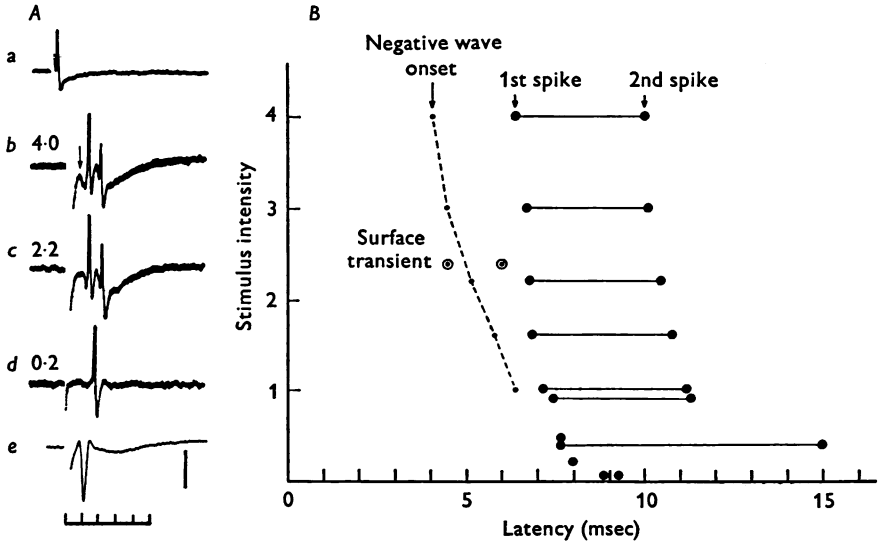


Fig. 3. Responses of mitral cell recorded at depth of 626 $\mu$m. *A*, extracellular recordings of unitary spikes. *a*, antidromic spike response to lateral olfactory tract volley. *b–d*, orthodromic spike responses to olfactory nerve volleys at indicated stimulus intensities (arbitrary units). *e*, olfactory nerve potential wave transient recorded at bulbar surface at 2·3 shock intensity. Positivity upwards, negativity downwards, in these and other recordings. Spikes retouched. Time scale in 5 msec divisions. Vertical bar: *a–d*, 2 mV; *e*, 4 mV.

*B*, graph of response latencies, measured from shock artifact, for different intensities of olfactory nerve shocks. Latencies are plotted for the onset of the negative wave recorded at this depth (see small arrow in *A b*), and the first and second spikes of the unitary response. Also shown are the onset and peak latencies of the surface olfactory nerve transient ⊙ in (*e*).

The problem of estimating the synaptic delay for orthodromic activation of bulbar units is well illustrated by this experiment. One estimate can be based on the interval between the onset of the unit spike and the onset of the evoked potential recorded at this depth (see small arrow in trace *b*, and

dashed line in graph *B*). It is apparent in both the traces and the graph that, with increasing shock strengths, the onset of the evoked potential shortened more than the onset of the spike. The interval between the two was about 1 msec at lower intensities, increasing to more than 2 msec at higher intensities. On the other hand, if the difference between the spike onset and the peak of the negative wave is measured, that interval remained at a relatively constant value of about 0·5 msec at all intensities.

Units varied in the extent to which the latency of the first spike changed with increasing shock intensity. We have found it convenient to use the term elastic to refer to responses which changed considerably, and inelastic to refer to responses the latency of which stayed relatively constant. These terms can refer to the absolute latency as calculated from the shock artifact, or relative to a component of the evoked potential. Elastic and inelastic properties have important implications for the mechanisms underlying the responses (see Discussion).

An estimate of synaptic delay may also be obtained by comparing the spike onset with the olfactory nerve-evoked transient recorded at the bulbar surface. The example shown in Fig. 3(*e*) indicates a delay, on this basis, of about 2·3 msec from the onset of the olfactory nerve transient, and about 0·8 msec from its peak, for this particular shock intensity (see (⊙) in Fig. 3*B*). This would be in analogy with the calculation of synaptic delays of motoneurones based on potentials recorded at the dorsal root entry zone (Brock, Coombs & Eccles, 1952). However, this method may not lead to an accurate calculation in the case of the olfactory bulb (see below and Discussion).

The responses in Fig. 3 are representative of the majority of units which responded with a spike during the period of the early brief negative wave of the evoked potential. This, together with the regularity of the responses in repeated trials, is consistent with the interpretation of a monosynaptic connexion from the activated olfactory nerves on to such units.

A unit which responded with a long latency spike is illustrated in Fig. 4*A*. This unit was driven antidromically from the lateral olfactory tract (trace *a*). The associated lateral olfactory tract waves (*b*) show that this unit was recorded in the deep external plexiform layer, and could therefore be either a slightly displaced mitral cell body, a mitral cell dendritic trunk, or a deep tufted cell body. The olfactory nerve response consisted of a single spike at all intensities; as can be seen in traces (*c–f*), the spike occurred near the peak of the late slow negative wave of the evoked potential.

The latencies of the spike and the evoked potential waves are plotted in the graph of Fig. 4. It can be seen that relative to the first negative wave (filled circles in graph on right) there was a shortening of the latency at

higher stimulus intensities. The latency of this spike may reflect a long synaptic delay in a monosynaptic pathway from olfactory nerves, or a polysynaptic pathway through circuits within the glomerular layer. It is important to note that the long latency is not explained by the possibility that this unit was situated some distance 'off-beam' from the activated olfactory nerves. The sharp evoked potentials give assurance that this unit was within the active region.

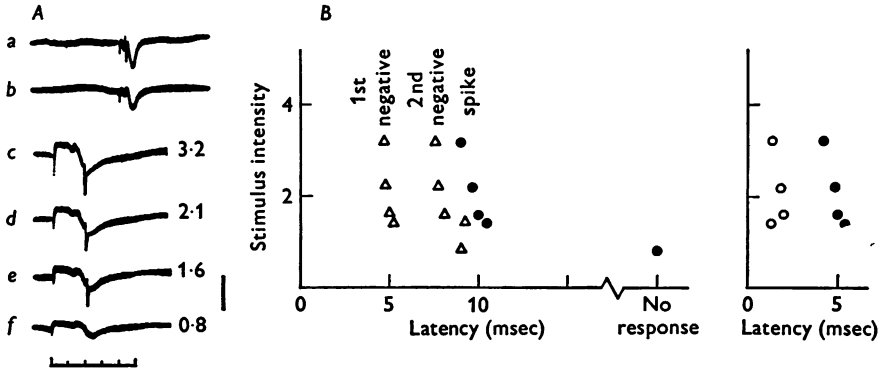


Fig. 4. Responses of mitral cell recorded at depth of 722 $\mu$m. *A*, extracellular unit recordings. *a–b*, threshold antidromic response to lateral olfactory tract volley. *c–f*, orthodromic responses to olfactory nerve volleys, at indicated stimulus intensities (arbitrary units). Spikes retouched. Time in 5 msec divisions; vertical bar, 4 mV.

*B*, graph of response latencies measured from shock artifact, at different olfactory nerve stimulus intensities. Open triangles, latency of onset of first and second negative waves; filled circles, spike latency. Far right: spike latency measured from onset of first (filled circles) and second (open circles) negative waves.

The latency of onset of the second negative wave is also plotted in Fig. 4*B*. It can be seen that there was little or no significant change in the interval between the onset of this wave and the spike (open circles in graph on right). Although the recordings give the impression that the spikes arise from this wave, it is likely that this second wave is mainly generated by activity in deeper layers that is not related to the initial activation of bulbar units through the glomeruli (see Orrego, 1960; Nicoll, 1972*a*; Getchell & Shepherd, 1975).

The absolute latencies of all units in which this property could be systematically studied are summarized in Fig. 5. The three main recording depths are distinguished, and general categories of high and low threshold for eliciting a response. It can be seen that low threshold units were found at all levels, with similar latency distributions (though wider spread) in the external plexiform layer. High threshold units were also found at all

depths; the units in the glomerular layer and external plexiform layer had the shortest latencies and widest range. Summarizing in another way, there was a scatter of latencies at all depths; units with short latency as well as low threshold responses were found at all levels, with glomerular layer and external plexiform layer units showing a tendency toward the shortest latencies. In addition to these attributes, there was a tendency

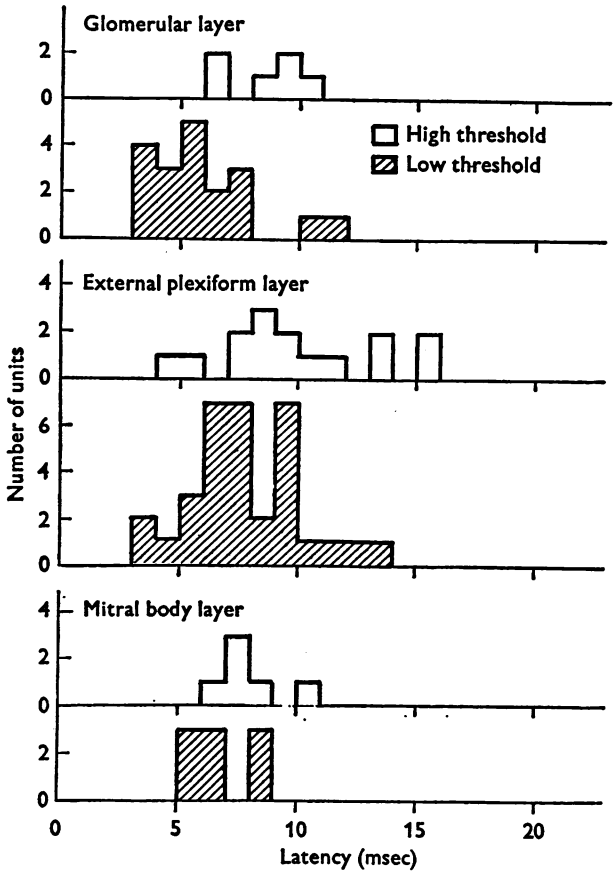


Fig. 5. Summary of responses of eighty-eight units recorded from three main layers in the bulb. Only units for which systematic data regarding recording depth, minimum spike latency, and relative threshold were obtained are included.

for units at the glomerular layer to be active spontaneously and respond repetitively. Finally, units at all levels were found which were not driven by volleys; some of these were spontaneously active and were suppressed by the volleys, others showed no effect.

*Effects of a conditioning volley on mitral cell responses*

*Facilitation.* In the analysis of effects of conditioning volleys, particular interest was focused on volleys at intensities that were near threshold for eliciting spike responses in the bulbar units. Fig. 6*A* shows the responses of a presumed mitral unit to paired shocks of equal intensity. It can be

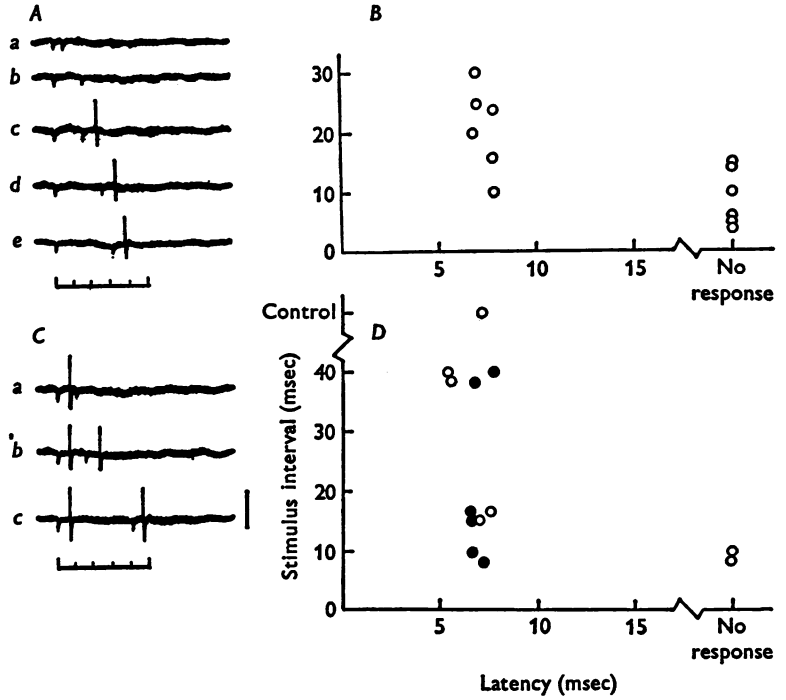


Fig. 6. Responses of mitral cell recorded at depth of 614 $\mu$m. *A*, extracellular unit recording. Paired olfactory nerve shocks were of equal intensity, just below threshold for eliciting a spike response when delivered singly. Note facilitation of response to test volleys in (*c–e*). Time in 10 msec divisions. *B*, graph of latency of spike response to test volley for different stimulus intervals. *C*, responses when shocks were increased slightly to just over threshold for eliciting a spike when delivered singly. Note blockage due to refractoriness in (*a*). Spikes retouched. Vertical bar, 4 mV. *D*, graph of latency of spike responses to conditioning (filled circles) and test (open circles) volleys.

seen that, whereas the unit failed to respond with a spike to the conditioning volley, it did respond to the test volley when stimulus intervals were longer than 5 msec. The results are plotted in Fig. 6*B*. The ability of the unit to respond to a subthreshold test volley may be referred to as facilitation, without implying a particular mechanism (see Discussion). The

failures to respond to the test volley at shorter intervals presumably reflect the refractory period of the olfactory nerves (Getchell & Shepherd, 1975).

When the intensity of the shocks was increased slightly to just over threshold for eliciting a response in single control trials, the results shown in Fig. 6*C* were obtained. It can be seen that the unit responded to the conditioning volley in every trial with a single spike, and that the responses at different intervals to the test volley were similar to those in Fig. 6*A*. These results are plotted in Fig. 6*D*. Note that at the interval of 40 msec the spike response to the test volley occurred at an earlier latency than in the control. This may also be considered as a property reflecting facilitation of the response.

The facilitatory effects of a conditioning volley on this unit were of medium duration. Subthreshold summation (as in Fig. 6*A*, *B*) was found to be maximal at 30–50 msec, declining gradually over a period of about 150 msec. Possible evidence for a small degree of long-lasting facilitation was the fact that, as testing was carried out over a period of 30 min, the threshold response changed from a single spike to two spikes. Effects due to the impingement of the pipette tip could not be ruled out, but it seemed likely that this slight increase in excitability could be due at least in part to a gradual build up of the facilitatory influence on this unit. Extreme degrees of facilitation, as in certain other central synapses (cf. Bliss & Lømo, 1973), were never seen in olfactory bulb unit responses to afferent volleys.

Another method for testing excitability changes is illustrated in the histogram of Fig. 7. Paired shocks were delivered to this unit at an interval of 20 msec. The shock intensity was just at threshold, i.e. there were responses in approximately half the trials, as can be seen for the controls in (*A*). In those trials in which a conditioning volley failed to elicit a spike, the test volley always elicited a spike, and at a shorter latency than the controls, as shown in (*B*). Thus, facilitation, in terms of both lower threshold and shorter latency, occurred. This was also found in the trials in which the conditioning volley succeeded in eliciting a spike (*C*). These results are similar to those previously reported for glomerular unit responses (Shepherd, 1971) except for the lack of effect of a conditioning spike in (*C*). The significance of these results will be discussed later and by Getchell & Shepherd (1975).

Facilitation as described here was a common finding in our experiments. However, it could only be brought out by weak volleys, near threshold strength for the unit under study. Of twenty-six units recorded at the mitral body depth, facilitation was found in ten, no facilitation could be demonstrated in seven and the rest were not adequately tested.

*Suppression.* Suppression of responsiveness of mitral units following

a conditioning volley was a common finding. It took several forms. The simplest was an increase in the latency of a spike response relative to the control value. This is shown in the recordings of Fig. 8 (insets *a*, *b*). Note that both conditioning and test spikes in these responses arose before the onset of any summed potential wave. The results are plotted in the graph of Fig. 8, and it can be seen that there was facilitation, in terms of an earlier latency for the test response, at low shock intensities just over threshold, whereas there was suppression, in terms of a longer latency, at higher shock intensities.

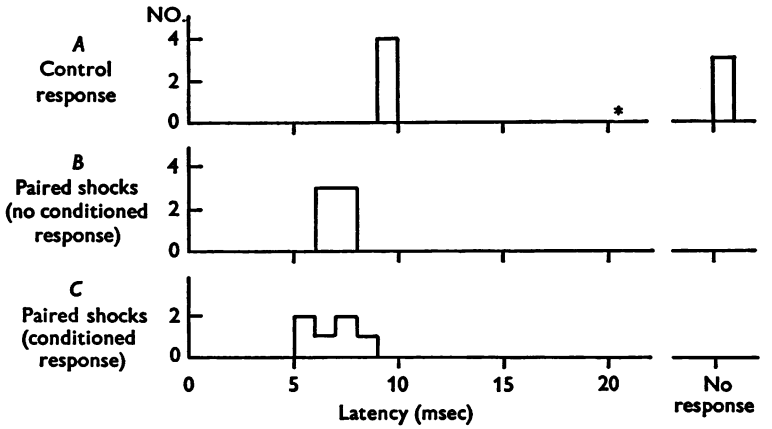


Fig. 7. Graphs summarizing test of excitability of presumed mitral cell (recording depth 668 $\mu$m) with paired olfactory nerve volleys at 20 msec interval. Shocks to olfactory nerve were of equal intensity. *A*, control for threshold response to test volley. Asterisk, second spike discharge in one trial. *B*, responses to test volleys when conditioning volleys did not elicit a spike. *C*, test responses when conditioning volleys elicited a spike.

The relation of the initial (unconditioned) responses of this unit (filled circles in Fig. 8) to the intensity of stimulation deserve further comment. The spike response at threshold had a very long latency, which decreased gradually with increasing intensity. A particular intensity was then reached (about $\times$ 3·5 threshold in Fig. 8) above which the latency remained the same. As explained above, we characterize such a response showing large latency changes as elastic, and those showing fixed latencies at different intensities as inelastic. Fig. 8 shows that a unit could show both types of behaviour in different parts of the intensity–response curve.

More profound types of suppression are illustrated in Fig. 9. Recordings of responses of this unit to volleys of increasing intensity are shown in the inset and the results are summarized in the graph. First to be described are the responses in the unconditioned bulb, i.e. the responses to the first volley

(filled circles). At the lowest intensities, just above threshold, the unit responded with a brief burst of impulses that began some 20–30 msec after the shock artifact (see inset, 0·6). At medium shock intensities (cf. inset, 2·1) the unit responded with a spike at a short latency (7–8 msec), and the later spike discharges became delayed in onset and briefer in duration. At the highest intensities the unit responded only with a single early spike (inset, 4.7). The delay and then absence of later repetitive

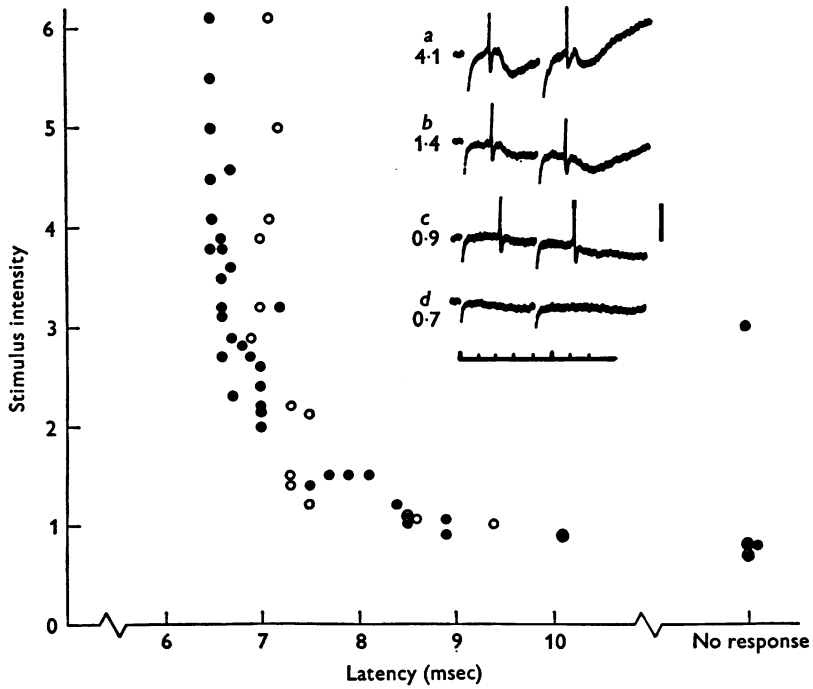


Fig. 8. Latencies of responses of mitral cell (recording depth 612 $\mu$m) to paired olfactory nerve volleys of different intensity. Spike responses to conditioning volleys, shown by filled circles, to test volleys, by open circles. An additional series of trials with single shocks is also included among the points plotted by filled circles, to demonstrate regularity of responses. Inset, representative recordings of spike response (*a–d*) to paired volleys at indicated intensities (arbitrary units). Spikes retouched. Time in 5 msec divisions; vertical bar, 4 mV.

discharge with increasing shock intensity is in itself evidence of suppression of excitability of this unit during that time period following the initial response. This effect has been seen in units at other levels in the bulb (see below, and Getchell & Shepherd, 1975), and it will be convenient to refer to it as curtailment of repetitive discharge.

The responses of this unit to test volleys are also plotted in Fig. 9 (open circles) and may be summarized as follows. At weak shock intensities there was evidence of subthreshold facilitation of an early spike response to test volleys. At medium intensities there were occasional early spike responses, but, more frequently, suppression, while at high intensities there was complete suppression of the later repetitive discharge.

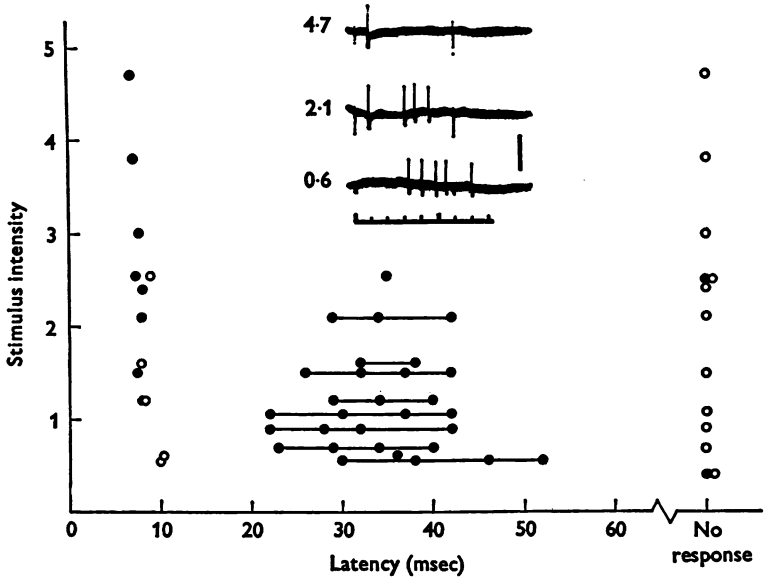


Fig. 9. Latencies of spike responses of presumed mitral cell (recording depth 776 $\mu$m) to paired olfactory nerve volleys of different intensity. Responses to conditioning volley shown by filled circles; note early single spike and later burst (connected by horizontal lines). Responses to test volley shown by open circles. Inset, representative recordings at indicated intensities (arbitrary units). Spikes retouched. Time in 10 msec divisions; vertical bar, 4 mV.

This suppression of repetitive discharges by a conditioning volley was typical of most units that gave this type of discharge. The complete suppression of a test response (both early and subsequent spikes) was also a common finding, in some units found throughout the entire range of intensities, in others only at certain intensities. Suppression was found in eighteen of the twenty-six mitral body layer units. Only one unit failed to demonstrate suppression to extensive testing with paired olfactory nerve volleys.

*Responses of external plexiform layer units*

Most of the properties described above for mitral units were also found in units recorded from the external plexiform layer, i.e. at depths in the range 400–600 $\mu$m (see Fig. 2). The responses of such a unit, recorded at a depth of 414 $\mu$m, are summarized in Fig. 10. With regard first to the

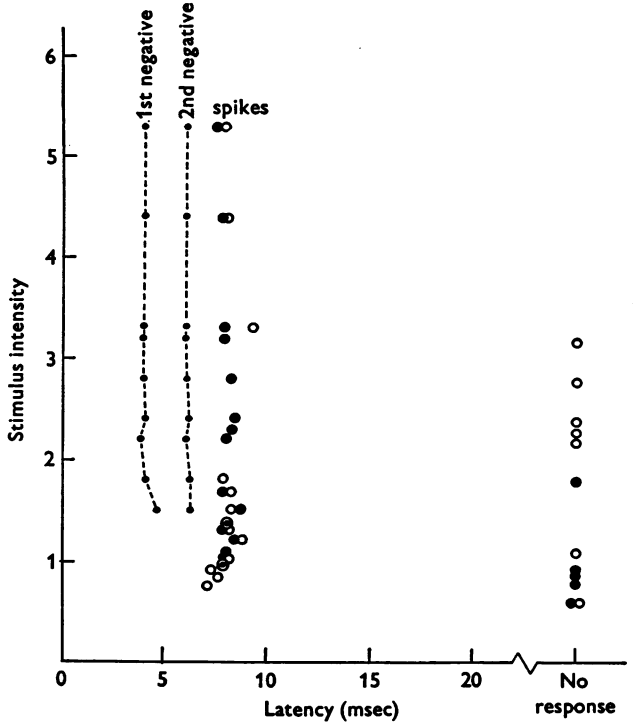


Fig. 10. Latencies of onsets of first and second negative waves and spike response of external plexiform layer unit (depth 414 $\mu$m) to paired olfactory nerve volleys of different intensity. Conventions as in previous figures.

responses in the unconditioned bulb (filled circles), it can be seen that this unit responded with a single spike at all shock intensities. The latency was similar at all intensities, i.e. this was an inelastic response as defined previously. The latencies of onset of the first and second negative waves recorded at this depth are also indicated on the graph. Relative to the onset of the first negative wave, the latency of the spike was approximately 4 msec. Despite this relatively long latency, the spike response shows little scatter in latency throughout the intensity series.

Paired volleys of equal intensity were delivered to this unit at an interval

of 40 msec. It can be seen that the response to the test volley (open circles) had a lower threshold than the response to the conditioning volley, and that the threshold response occurred at a shorter latency than it did at other intensities. Thus, there was facilitation in terms of both subthreshold summation and shortening of latency, similar to that described above for mitral units (see Figs. 6–8).

At higher shock intensities the behaviour of this unit passed through several types of response. Above the threshold region there was a level in which there was little or no difference between conditioning and testing response; above this was a level of complete suppression; finally, at the highest intensities, there was a delayed response. This was characteristic of many units, both in the external plexiform layer and in the mitral cell body layer (cf. changes from facilitation to suppression in Figs. 7 and 8). It is possible that these effects reflect the activation of different bundles of nerve fibres converging on this unit (see Discussion).

It is of interest to note that another unit was encountered at the same depth as the unit shown in Fig. 10. This other unit differed in its response properties: it gave a short latency response (about 1 msec after the onset of the first negative wave); it responded with a repetitive discharge at all intensities above threshold; and there was only minimal facilitation (at threshold) and suppression (at higher shock intensities) of the test response. Recordings of neighbouring units varied in these respects; some showed similar response characteristics, others were different, as in this case (see also Getchell & Shepherd, 1975).

The time course of suppression of the response to a test volley in another external plexiform layer unit is illustrated in Fig. 11. In *A* are shown the recordings of the responses; the data are plotted in *B*. It can be seen that complete suppression of the test response began at 25–40 msec interval and lasted until 70–80 msec. Even when a response occurred it consisted of only a single spike compared with the double discharge of the control response. A long latency of onset of suppression was a common finding and has important implications (see Discussion).

### *Giant spikes and intracellular recordings*

Unitary spikes which grow to large amplitudes in the absence of significant changes in base line DC potential level are termed 'giant extracellular spikes' (Granit & Phillips, 1956), and are especially common in recordings from the olfactory bulb (Phillips *et al.* 1963). An example is shown in Fig. 12. This unit displayed a tendency to fire impulses in bursts. A burst was triggered by lateral olfactory tract volley in (*a*) and also occurred as a spontaneous discharge in (*b*), in which case there was only a single spike of small amplitude in the subsequent response to the lateral olfactory

tract volley (*b*). Note that in both cases there was a progressive decrease in spike amplitude during the burst. There was also a hesitation on the upstroke of the spike, which became more prominent in successive spikes. This appears similar to the A–B separation in motoneurone spikes, believed to signify sequential impulse generation in the initial segment (A) and axon hillock or cell body region (B) (see Eccles, 1957; Fuortes, Frank & Becker, 1957). A–B spikes have previously been described in giant spikes recorded from cell bodies of mitral cells (Phillips *et al.* 1963). It is reasonable to conclude that the recording site for the unit in Fig. 12 was also a cell body, and the recording depth of 498 $\mu$m suggests that the type of cell was a tufted cell or a displaced mitral cell.

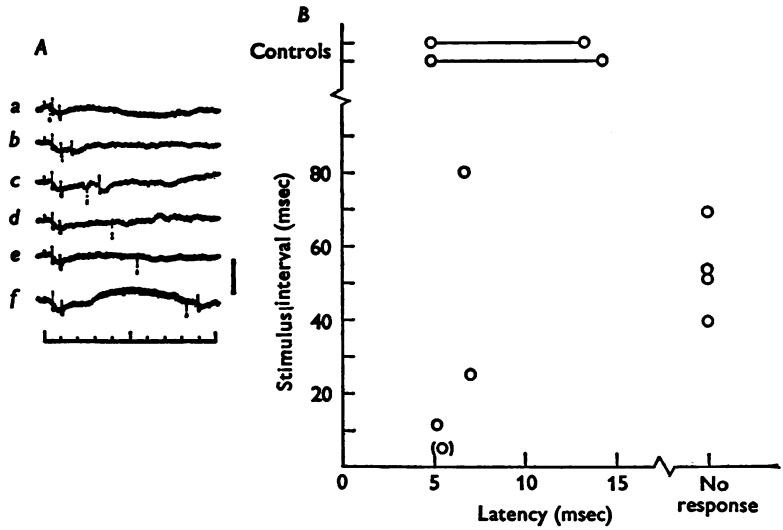


Fig. 11. External plexiform layer unit (depth 436 $\mu$m) tested with paired olfactory nerve volleys at different intervals. *A*, recordings of spike response; note successful test responses (*a–c, f*) and suppression (*d, e*). Time scale in 10 msec divisions; vertical bar, 2 mV. *B*, graph of latencies of test spike at different shock intervals. Control responses (double spike discharge) plotted above.

A trial with an orthodromic volley, followed by an antidromic volley, is shown in (*c*). It can be seen that a spontaneous impulse (the first spike on the trace) had no effect on the latencies of the initial or subsequent spikes in the burst. Apparently the spontaneous impulse did not spread to the site of orthodromically induced synaptic input in the dendrites. The subsequent antidromic test response was suppressed.

A series of trials with strong olfactory nerve conditioning volleys and weaker olfactory nerve test volleys is illustrated in (*d–f*). Note the A–B

hesitations in the later spikes of the burst discharges, and the constant number of spikes in the bursts (cf. Shepherd, 1963*b*, 1970). The ability of the second, weaker, volley to elicit a spike in (*d*) is evidence that the site of orthodromic synaptic input is to some extent isolated from inhibitory circuits in the bulb (see Discussion).

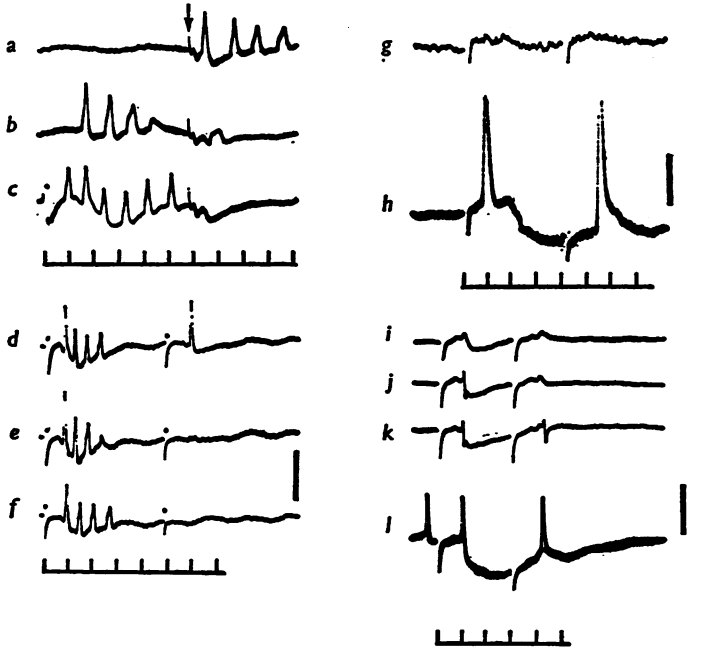


Fig. 12. *a–f*, giant spikes, recorded extracellularly, from unit at 498 $\mu$m depth. Olfactory nerve shock shown by small dot, lateral olfactory tract shock by arrow. Time: (*a–c*) 5 msec divisions, (*d–f*) 10 msec divisions. Vertical bar, 10 mV.

*h*, intracellular recording from unit at 424 $\mu$m recording depth. Paired olfactory nerve volleys: note the first spike is spontaneous (see text). Membrane potential, 30 mV. *g*, extracellular field potentials after unit lost. Time in 5 msec divisions. Vertical bar: 10 mV for *h*; 2 mV for *g*.

*l*, intracellular recording from unit at 656 $\mu$m recording depth: antidromically driven. Paired olfactory nerve shocks; note early spontaneous spike followed by spike responses to volleys. Membrane potential undetermined. *i–k*, extracellular recordings of same unit to paired olfactory nerve volleys of increasing strength. Time in 5 msec division; vertical bar, 2 mV.

An intracellular recording from a different unit is shown in (*h*); the recording depth of 424 $\mu$m suggests that this was a tufted cell, or possibly a large mitral cell dendrite. The trace in (*h*) was particularly fortunate, for it shows that when this unit was tested with paired olfactory nerve volleys,

a spike occurred at a latency of only 3·0 msec after the first shock artifact; this was too brief for orthodromic activation to have occurred in this unit, and the spike must therefore have been a spontaneous discharge. At 9·0 msec latency an abrupt hyperpolarization of the membrane occurred. At the peak of the hyperpolarizing wave the second volley was set up, and the spike occurring 6·5 msec later was clearly a response to this volley. Note that this spike arose sharply and reached a larger amplitude than the first spike; it also had a prolonged falling phase and a small after-hyperpolarization. The extracellularly recorded potentials after this unit was lost were of very small amplitude (*g*).

Recordings from another unit at the layer of mitral cell bodies (depth 656 $\mu$m), are shown in (*i–l*). The extracellularly recorded unitary spike responses to paired olfactory nerve volleys are shown in (*i–k*). Penetration then occurred, and the trace in (*l*) illustrates the intracellularly recorded response properties of this cell. A spontaneous spike had no effect on the subsequent responses. The spike response to the first volley arose sharply from base line, and was immediately followed by a large hyperpolarization. The spike response to the second volley was delayed and arose from a slow depolarization. There was a prolonged falling phase after the second spike, followed by a small hyperpolarization.

## DISCUSSION

### *Recordings from mitral cell dendrites*

The depth distribution of recorded units (Fig. 2) is in good correlation with the distribution of mitral, tufted and periglomerular cells, with the exception that the large number of units recorded in the middle and deep parts of the external plexiform layer is not in accord with the paucity of cell bodies at those levels. This was not due to inaccuracies in the depth measurements. It suggests rather that some of the recordings must have been made from the other large elements of the external plexiform layer, the dendritic trunks of mitral cells. This is consistent with evidence that many units in the external plexiform layer can be driven antidromically from the lateral olfactory tract with short latencies similar to those of mitral cells recorded at the layer of mitral cell bodies (Shepherd, 1962; Nicoll, 1970; Shepherd, 1972, 1975). These units are probably not tufted cell bodies, because tufted cells have thinner axons than the mitral cells, and therefore spikes recorded from them would be expected to have slower conduction velocities and consequently longer latencies when elicited antidromically from the lateral olfactory tract.

The question of spike generation in mitral cell dendrites has been discussed by Rall & Shepherd (1968) and Shepherd (1972). Physiological

studies have given no evidence for prepotentials that might signify sites of active impulse generation such as reported for hippocampal pyramidal neurones (Spencer & Kandel, 1961) and cerebellar Purkinje cells (Llinas & Nicholson, 1971). Biophysical analysis (Rall & Shepherd, 1968) has suggested that the mitral dendritic tree has a relatively short electrotonic length. As a consequence, an impulse that is set up in the soma can spread with only moderate decrement through the dendrite by passive means alone. Such spread would be consistent with our findings of short latency, lateral olfactory tract driven unit spikes in the external plexiform layer, and the fact that spike amplitudes of such units are smaller the more superficially they are recorded in the external plexiform layer (Shepherd, 1962, 1975).

*Monosynaptic inputs to mitral and tufted cells*

The precise determination of the synaptic delay for transmission from olfactory nerves to mitral cells has been a difficult problem. The mitral cell responses can be measured relative to the olfactory nerve transient recorded at the bulbar surface, as described in Results. The limitation of this method is that this transient in some preparations is indistinct or absent, in spite of other indications of an 'on beam' recording site. Also, the surface transient is weighted for the nerve fibres at the surface, and these most likely are destined for more posterior parts of the bulb, rather than the site of unit recording deep to the surface (cf. Freeman, 1974*a*). Nor is it clear which component of the triphasic nerve transient is relevant for calculation of synaptic delay. Finally, unit responses which by other criteria (short absolute latency, unvarying with stimulus intensity) strongly suggest a monosynaptic input, may occur at a latency of up to 3 msec after the onset of the nerve transient, i.e. much more than expected if the monosynaptic delay is similar to that in motoneurones (cf. Eccles, 1964), even taking account of conduction times in the nerve.

The latency of a mitral unit response can also be measured relative to the onset of the evoked potential recorded at that depth. However, this also leads to inconsistencies. For example, increasing shock strength should cause a unit to fire with briefer latencies. In Fig. 2, the unit fired with a briefer latency, relative to the shock artifact, yet the latency relative to the onset of the evoked potential actually increased. In other cases, the spike response was found to occur at or before the onset of any detectable evoked potential (Fig. 8).

We conclude that physiological evidence for a monosynaptic connexion in the experiments in the olfactory bulb has to be based on one or more of several criteria: minimal spike latencies; short latency relative to the olfactory nerve transient (or latency relative to the evoked potential);

inelastic latency (unvarying with volley intensity); fixed latency and stability of response with repeated trials. Given these criteria, spikes occurring up to 5–10 msec after the arrival of a volley in the bulb could possibly be consistent with the interpretation of a monosynaptic input. In some cases (i.e. Fig. 8) the spike latencies were long with weak volleys, becoming shorter with stronger volleys in a continuously graded manner. We have termed this response type 'elastic'. Apparently, in these units, the stronger shocks excited more olfactory axons and there was convergence of these axons into the glomerulus to which the dendrite of the cell was connected. The shortening of the spike latencies implies a larger and faster rising e.p.s.p. due to convergence of larger numbers of olfactory axons.

On the other hand, some units responded with a spike which had a fixed latency at all shock intensities, i.e. the latency was as brief with weak, just threshold volleys, as with volleys many times threshold (cf. Fig. 10). We refer to these responses as 'inelastic'. Apparently these units receive input from a specific population of olfactory nerves. With the stimulating electrode situated so as to stimulate this population at weak intensities, the activation of more axons by stronger shocks provided no further input into this unit.

We therefore conclude that single volleys set up monosynaptic e.p.s.p.s in many mitral and also tufted cells. This is consistent with the anatomical evidence for synapses from the olfactory nerves on to mitral and tufted cells in the rabbit (Pinching & Powell, 1971*b*; White, 1972, 1973; Willey, 1973). The morphology of the synapses (asymmetric membrane densities and spherical synaptic vesicles) is consistent with the excitatory action revealed by the present and previous physiological studies (Shepherd, 1963*a*, *b*; Yamamoto *et al.* 1963). The difference between elastic and inelastic responses may reflect the differences between diffuse and grouped projections of olfactory nerve fibres to glomeruli, as revealed by recent anatomical studies (Land, Eager & Shepherd, 1970; Land, 1973; Land & Shepherd, 1974; see Getchell & Shepherd, 1975).

It further appears that there are differing time courses of the e.p.s.p.s in different mitral cells situated in the path of an olfactory nerve volley. This conclusion, based on extracellular unit recordings, is also supported by results of intracellular recordings. Yamamoto *et al.* (1963) reported intracellular recordings in which some presumed mitral cells responded with a slow e.p.s.p. while others responded with a spike preceded by little or no discernible e.p.s.p. Our present intracellular recordings support their result (Fig. 12).

A slow rising phase of the e.p.s.p. in mitral cells would be significant for the relation between mitral and periglomerular cells. There is clear anatomical evidence for numerous synaptic connexions from mitral and tufted

cells on to periglomerular cell dendrites within the olfactory glomeruli (Pinching & Powell, 1971*b*; White, 1972, 1973). Fig. 5 shows that glomerular layer units tend to have slightly shorter spike latencies than do mitral cells. This does not mean necessarily that mitral cells have longer synaptic delays, or additional synaptic relays from olfactory nerves. The difference could well be a function of the rising phase of the e.p.s.p. One should note in this regard the relatively longer distance between the sites of the e.p.s.p. and of impulse initiation in the mitral cell than in the periglomerular cell. The periglomerular dendritic tuft has smaller diameter processes, which might be expected to generate larger and faster rising amplitude e.p.s.p.s than those in the mitral dendritic tufts, by virtue of higher input resistances (see Rall & Rinzel, 1973).

### *Facilitation and suppression*

The experiments with paired volleys have given clear evidence of both facilitation and suppression of mitral and tufted cells. Evoked potential studies using strong volleys have revealed only suppression of testing responses (Orrego, 1960; Nicoll, 1972*a*; Freeman, 1974*c*). It appears that unitary recordings and the use of weak volleys may be necessary for revealing facilitation. The facilitation has taken several forms: shorter spike latency after the second volley, lower spike threshold, subthreshold summation, and potentiation of spike discharge (increased frequency and duration). The duration of these effects was usually 20–30 msec. One possibility is that this reflects the time course of e.p.s.p. elicited in these cells by the conditioning volley.

The facilitation observed in our study appears to be much weaker than the powerful and long-lasting potentiation of evoked potentials reported in certain other regions of the C.N.S. (e.g. dentate fascia, Bliss & Lømo, 1973; optic tectum, Chung, Bliss & Keating, 1974). Unfortunately, meaningful comparison between the results obtained with evoked potentials and unitary responses in these different regions does not yet seem possible.

Suppression has also taken several forms: longer spike latency, raised threshold, complete suppression of spike response and depression of spike discharge frequency or duration. These findings are consistent with properties of inhibitory post-synaptic potentials (i.p.s.p.s) in other neurones (Eccles, 1964; Purpura, 1972). Suppression of mitral and tufted cell responses characteristically began 10–20 msec after the arrival of a volley, and could last up to several hundred msec.

Inhibition of projection neurones is commonly mediated by interneurones in many parts of the nervous system. In the olfactory bulb, there are two main types, as explained in the Introduction: granule cells and

periglomerular cells. Inhibition mediated by granule cells on to mitral cells has been demonstrated in experiments using paired antidromic volleys (Phillips *et al.* 1963; Rall & Shepherd, 1968), and similar inhibition of antidromic invasion is produced by a conditioning orthodromic volley (Shepherd, 1963*a*). The pathway for this inhibition, through reciprocal dendrodendritic synapses between mitral and granule cells, has been documented elsewhere (Rall *et al.* 1966; Rall & Shepherd, 1968; Nicoll, 1969; Shepherd, 1972).

In the present experiments we have been primarily concerned with the inhibition revealed by paired orthodromic volleys. In this case, suppression of the response to a test volley could be due to synaptic inhibition mediated through the granule cells, as just described, or through the other type of interneurone, the periglomerular cell. In the next paper (Getchell & Shepherd, 1975) evidence will be presented for periods of facilitation and suppression elicited by paired orthodromic volleys in periglomerular cells that are similar to those reported here for mitral and tufted cells. This will suggest that the facilitation and at least part of the suppression reported here in mitral and tufted cells is due to dendrodendritic and axodendritic synaptic interactions through periglomerular cells at the glomerular level.

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