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RESPONSES OF MITRAL CELLS TO STIMULATION OF THE LATERAL OLFACTORY TRACT IN THE RABBIT

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Our general picture of neuronal function is derived largely from intracellular studies of the spinal motoneurone (Eccles, 1957; Fuortes, Frank & Becker, 1957), and needs to be kept under review as fresh evidence is gathered from central neurones of other types, for example, the cerebellar Purkinje cell (Granit & Phillips, 1956), the Betz cell (Phillips, 1959, 1961) and the hippocampal pyramidal cell (Kandel, Spencer & Brinley, 1961). In these cases selective electrical stimulation of afferent and efferent pathways is not so easily accomplished as with motoneurones. From this point of view the mitral cells of the rabbit's olfactory bulb offer certain advantages. Here the afferent pathway (the olfactory nerves) is clearly defined and completely separated from all but the terminal dendritic tufts of second-order mitral cells. The mitral cell bodies lie mostly in a single layer; the shafts of their primary dendrites are apparently free of synaptic contacts, their secondary dendrites and recurrent axon collaterals terminate in distinct histological laminae, and their axons form a distinct efferent pathway (the lateral olfactory tract).

Impulses in the lateral olfactory tract, set up by electrical shocks, invade mitral cells antidromically (Phillips, Powell & Shepherd, 1961; von Baumgarten, Green & Mancia, 1961; Yamamoto, 1961), and also elicit distinctive wave patterns in different histological layers of the bulb, permitting localization of the recording tip at the time of experiment (Phillips *et al.* 1961). The details of this method of localization will be presented, followed by an analysis of antidromic impulse generation in mitral cells, and the long-term changes in their excitability consequent on volleys in the lateral olfactory tract.

A disadvantage in this work was the inability to obtain satisfactory intracellular recordings. However, 'giant' extracellular spikes (Granit & Phillips, 1956) have been regularly obtained, and have allowed indirect

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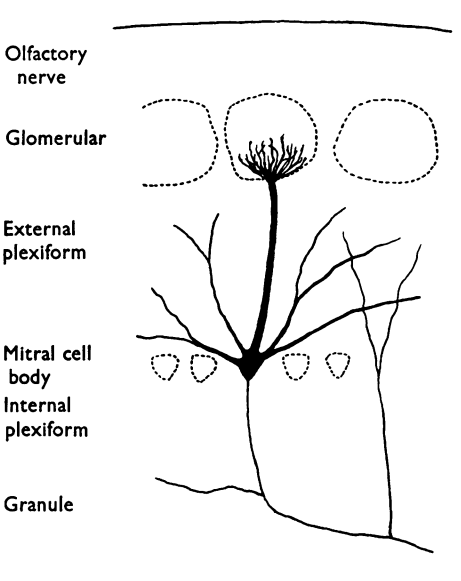
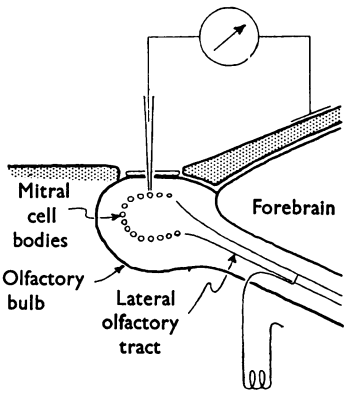
comparisons with intracellular studies in other neurones. Subsequent papers deal with the orthodromic responses of mitral cells, and the unitary activity of other cells within the bulb (Shepherd, 1963*a*, *b*).

Morphology of the mitral cell

According to Cajal (1911), the mitral cell bodies are mostly arranged in a layer 1–3 cells thick within the olfactory bulb (Text-fig. 1). Arising from the cell body are two types of dendritic process, primary and secondary. The primary dendrite is remarkable for its thickness, its smooth contours, and its perpendicular direction to the periphery. It ends in the interior of a glomerulus by a tuft of fine, free ramifications, in intimate contact with the terminal arborizations of olfactory nerve fibres. Secondary dendrites are several in number and, dividing repeatedly, proceed into the external plexiform layer. Their terminal branches form, with other neuronal processes, a dense plexus in this layer, without ever attaining the glomerular boundary.



Text-fig. 1



Text-fig. 2

Text-fig. 1. Diagram of a mitral cell, after Cajal (1911). Histological layers in the olfactory bulb are indicated on the left.

Text-fig. 2. Experimental arrangement for micropipette recording in the olfactory bulb and stimulation of the lateral olfactory tract of the rabbit.

From the deep surface of the cell body the axon arises. It crosses the internal plexiform layer *without* emitting collaterals and, reaching the granule layer, turns posteriorly to form part of the white matter of that zone. In its horizontal course the axon gives off collaterals, some ending in the granule layer, and some travelling peripherally to branch and end in the external plexiform layer.

At the postero-lateral border of the bulb the mitral cell axons converge to form the lateral olfactory tract; there are also small superior and internal pathways to the frontal pole of the cerebrum and to the olfactory tubercle respectively. The lateral olfactory tract

(see Text-fig. 2) is visible to the naked eye as a white band approximately 1 mm wide, which courses over the surface of the olfactory peduncle and prepyriform cortex, emitting a profusion of collaterals to these regions. The arborizations of these collaterals form a dense plexus, confined (almost exclusively in the rabbit) to the molecular layer of the underlying cortex, and making synaptic contact there with dendrites from deeper-lying pyramidal and polymorphic cells. Except for noting that, according to Cajal (1911), centrifugal fibres are present in the lateral olfactory tract, further histological details are beyond the scope of the present study.

METHODS

Preparations. The experiments were performed on 107 rabbits, with body weights between 1·5 and 2·5 kg. In the majority of animals this meant an age of 2–4 months; in others the age ranged up to 1 year. Young animals were preferred because of the high incidence of atrophic rhinitis in rabbits, which leads to atrophy of olfactory nerves and transneuronal degenerative changes in the olfactory bulb (Matthews & Powell, 1962).

Anaesthesia. The animals were anaesthetized by the intravenous injection of a mixture of 10 % urethane (ethyl carbamate) and 1 % chloralose, in mammalian Ringer's solution. Routine tracheotomy was performed. For further surgical procedures, chloroform and oxygen could be delivered through the tracheal cannula, thereby avoiding any effects on the olfactory mucosa. The concentration of chloroform could be controlled between 0·5 and 3·0 %. It was terminated as soon as the operation was completed. Supplementary anaesthesia during the experiments consisted of intraperitoneal injections of either 1–2 ml. urethane–chloralose mixture, or 0·3 ml. Nembutal (sodium pentobarbitone; Abbot Laboratories). In both cases anaesthesia was kept moderately light, and the results did not appear to be differentially affected by these régimes. Rectal temperature was maintained between 35 and 40° C; deliberate hypothermia to 30° C in several preparations produced no clear effect on unitary activity.

Surgical procedures consisted of the removal of the left eye after ligature of the optic nerve and associated vessels. A small plate (cut from X-ray film) was pressed down on to the remaining orbital contents by a thin Perspex strut wedged across the orbit. This controlled any oozing from soft tissues, and adequately exposed the medial surface of the orbit. With a dental drill a small craniotomy opening was made over the lateral olfactory tract where it crosses the prepyriform cortex. A circular craniotomy opening of 6 mm diameter was also trephined directly over the dorsal surface of the left olfactory bulb, and the dura reflected (see Text-fig. 2).

Micro-electrodes. The micro-electrode holder, its hydraulic driving system, and the massive stand have been described elsewhere (Phillips, 1959). The micropipettes were made in a Schuster microforge from clean Pyrex glass capillaries of 4 mm diameter. The shaft diameter could be varied, and over the final 1·0 mm a uniform taper from about 30 μ was found to be optimal. They were filled by gentle boiling in 3 M-KCl solution under reduced pressure. The d.c. resistance was 5–30 MΩ, 15 MΩ being an optimal value. Potentials were recorded between the pipette tip and a chlorided silver plate under the skin at the back of the head.

Recording. Conventional micropipette recording techniques were used, with a cathode follower of the Nastuk & Hodgkin (1950) type. Grid current was 3×10^{-11} A; input capacity was 3 pF. The other half of the 12AY7 input valve was used for calibration. One channel to the Tektronix 502 amplifier-oscilloscope was capacitor-coupled, with a time constant of 0·5 sec, for high-gain recording. The other channel was directly coupled for low-gain recording and monitoring of resting d.c. potential level.

To restrict cardiovascular and respiratory pulsations in the bulb a circular disk (of X-ray film), slightly smaller in diameter than the craniotomy opening, was pressed lightly on to its dorsal surface. Holes 0·3 mm in diameter were punched in the disk, through which

the micropipette could be inserted. The tip passed easily through the pia-arachnoid without need for prior incision.

Stimulating. The paired stimulators have been described elsewhere (Kay, Phillips & Teal, 1958). Pulse voltage, duration and delay could be independently varied; repetitive trains could be delivered up to 600/sec. The stimulating current was measured by recording the voltage across a 10 kΩ resistor in series with the preparation. The shocks, isolated from earth, were delivered through a fine enamelled silver wire (diameter 80 μ). From one side of the terminal 1 mm of the insulation was scraped off, the exposed part lightly chlorided, and the wire shaped to ride gently on the surface of the lateral olfactory tract, about 11 mm from the recording site in the bulb (Text-fig. 2). This focal electrode was fixed to a support, adjustable in three planes, which in turn was mounted on the head holder; the support and holder were made by Dr E. H. J. Schuster. Focal shocks were cathodal, of 60 μsec duration, delivered generally at 1/sec. The indifferent electrode dipped into the Ringer's fluid filling the orbit.

Controls. Both wave and unitary responses in the bulb ceased after transection of the lateral olfactory tract between the point of stimulation and the bulb. The possibility of stimulus spread to nearby structures will be discussed later. In some experiments the frontal poles were transected by suction. Since the primary aim of this procedure was either to reduce intracranial pressure, or to reduce or effectively eliminate respiratory pulsations in the bulb, anatomical controls were not done. However, several gross post-mortem examinations showed complete removal of the anterior commissure and varying amounts of its rostral limbs. The removal always spared the most ventral tissue overlying the anterior cerebral vessels supplying the bulb and prepyriform cortex (Shellshear, 1920). The results obtained after these procedures suffered no apparent change in character, indicating that (1) blood supply to the recording and stimulating sites was not compromised (this was verified by microscopical observation of small vessels during an experiment) and (2) the structures resected (anterior commissure and most of the frontal pole) or isolated (contralateral olfactory bulb) do not contribute appreciably to the results obtained in intact preparations.

Anatomical procedures. To obtain a correlation between potential wave responses and bulbar laminae an extension of the method of Powell & Mountcastle (1959) was used. Depth readings were taken for each distinctive wave pattern during a micropipette penetration. Two such penetrations, in a transverse plane in the anterior bulb, were made; the pipettes were then thrust through the bulb, the shafts clipped off some distance above the surface, and left *in situ* (Pl. 1*A a*). As Powell & Mountcastle (1959) showed, a sufficient depth of penetration is necessary if the tracks are subsequently to be found in histological sections. With the same orientation and in a parallel transverse plane, two guide pipettes (*c*) were inserted in the posterior bulb. The lateral surface of the bulb was then exposed, and a pair of guide pipettes, in the same transverse plane, was inserted horizontally (*b*). The inter-electrode distance, at the bulb surface, was measured from micrometer readings for each pair of pipettes. The animal was killed and perfused with formol saline, and the entire bulb was removed and fixed.

After fixation, the bulb was carefully transected with a razor blade along the vertical guide electrodes under a dissecting microscope. All pipettes were removed, and Indian ink injected along the tracks left by the guide electrodes. After embedding in paraffin, serial sections were cut in the vertical plane parallel to the tracks. All the sections were mounted and stained with thionin. In favourable preparations the two horizontal guide tracks (*b*) and the two recording pipette tracks (*a*), respectively, could be found on one or two sections. By measuring the distance separating the horizontal guide tracks and comparing it with the previous experimental reading, a factor was obtained which allowed the experimental depth readings at which the potential wave responses were recorded to be correlated with the depths of the laminae below the bulb's surface, as seen in the histological section of that particular micropipette track (see Pl. 1*B*).

In the first attempts at this correlation only the vertical guide electrodes were used, since it was expected that they would allow a sufficiently accurate adjustment to be made for the usual shrinkage due to formalin fixation. It was eventually realized that just as important a factor in the correlation was the effect of the microtome blade on the tissue block during *vertical* sectioning. The horizontal guide electrodes were therefore employed to allow correction for this factor. Distortions during the cutting of sections are well known (cf. Olszewski, 1952). This distortion is of importance in dealing with small sections, as in the present case where the total distance under study was less than 1·0 mm. Among other sources of error, a careful watch was kept for 'tissue lag' during the pipette penetration. The surface reading was open to a small error, as were the individual determinations of the depths of each potential wave response pattern.

RESULTS

The localization of laminae

As a micropipette is inserted gradually through the dorsal surface of the bulb, while shocks are delivered to the lateral olfactory tract, a succession of potential wave responses is recorded (Phillips *et al.* 1961). A typical sequence is shown on the right of Pl. 1 *B*. At the surface, following the shock artifact, there is an initial sharp positive wave, followed by a brief negative wave, and then a second negative wave of longer duration. As the penetration proceeds deeper these wave patterns undergo a regular sequence of changes. The initial positive wave decreases in amplitude and disappears. The first brief negative wave increases in amplitude to a maximal value. The second negative wave increases to a maximal amplitude, and then gives place to two positive waves, the first brief, and the second of long duration and large amplitude. In the bottom two records there is a final trough of negativity.

Although minor variations were always present, essentially these same wave response patterns were seen in 65 micropipette penetrations carried out in this way. Since these potential waves gave such reproducible patterns, it appeared likely that they might bear some equally reproducible relation to the histological layers of the bulb. In Pl. 1 *B* a histological section of this particular penetration has been prepared in the manner described in Methods, and is juxtaposed to the wave responses. The changes in the waves can thus be correlated with the passage of the micropipette through the successive laminae. It can be seen that:

1. The initial positive wave, recorded at the surface, decreases in amplitude and has disappeared at the inner boundary of the external plexiform layer.

2. The first negative wave grows to reach a maximum in the granular layer.

3. The second negative wave reaches its maximum in the external plexiform layer.

At about 0·7 mm from the surface of the bulb, which is the approximate depth of the layer of mitral cell bodies, a shift of about 50 μ causes a large change in the potential wave pattern. The second negative wave becomes abruptly positive. It is tempting to suppose that this change depends on the crossing of the layer of mitral cell bodies. The positivity reaches its maximum when the pipette has penetrated well into the granular layer. Although many such penetrations gave similar results, such a conclusion needs further evidence, because a distance of only 50 μ is probably near to the limits of error of the method. Two further correlation experiments provided confirmation of the critical relationship between the inversion of the second negative wave and the crossing of the layer of mitral cell bodies.

In the experiment shown in Pl. 2 the micropipette was inserted at the lateral margin of the bulb. The recordings on the right show that, as the penetration proceeded, the two negative waves increased in amplitude; at the deepest point the first negative wave had begun to diminish. There was no reversal to large positive waves at any point in the penetration. In the histological section on the left the track of the micropipette is seen to pass tangentially through the external plexiform layer without penetrating the layer of mitral cell bodies.

In another experiment (Pl. 3) the micropipette was inserted near the medial aspect of the bulb, and the recorded wave potentials are at the left. The upper sequence shows the development of the second negative wave and its reversal to two positive waves. The maximal amplitude reached by the positive waves is seen in the single middle record. As the penetration proceeded, the positive waves diminished, and, in the lower set of records, reversed back to the two negative waves. The photographs (*A–D*) show the successive sites of the track of the micropipette on adjoining sections of the olfactory bulb. In this penetration the micropipette pierced the mitral cell body layer, and, after a short traverse of the granule layer (*B*), pierced the mitral cell body layer again before passing tangentially down the external plexiform layer (*C* and *D*).

These experiments confirm that the reversal of the second negative wave to a positive wave occurs at or near the layer of mitral cell bodies. It will also be noted that the wave patterns in lateral, ventral and medial aspects of the bulb were essentially similar to those found in the dorsal layers.

The identification of mitral cell responses

From the correlation of potential wave patterns with histological laminae it was possible to localize unitary activity when it was encountered during an experiment. At or near the mitral cell body layer (as determined by the reversal of the second negative wave to the positive waves) it was

relatively easy to pick up unitary spikes which responded singly to each shock to the lateral olfactory tract. With occasional slight adjustment of the depth of the micropipette tip a spike often grew to a considerable amplitude. Such a case is illustrated in Text-fig. 3*a*. In the upper sweep the first shock artifact is followed by an all-or-none spike with positive-negative conformation. Its latency is brief and the spike onset is abrupt. The peak-to-peak amplitude is 17 mV, remaining around that value for over an hour. Except for the single response to each tract shock there was only infrequent spike activity in this unit. The resting d.c. potential level did not shift appreciably throughout the recording period, indicating that the recording tip never entered the cell.

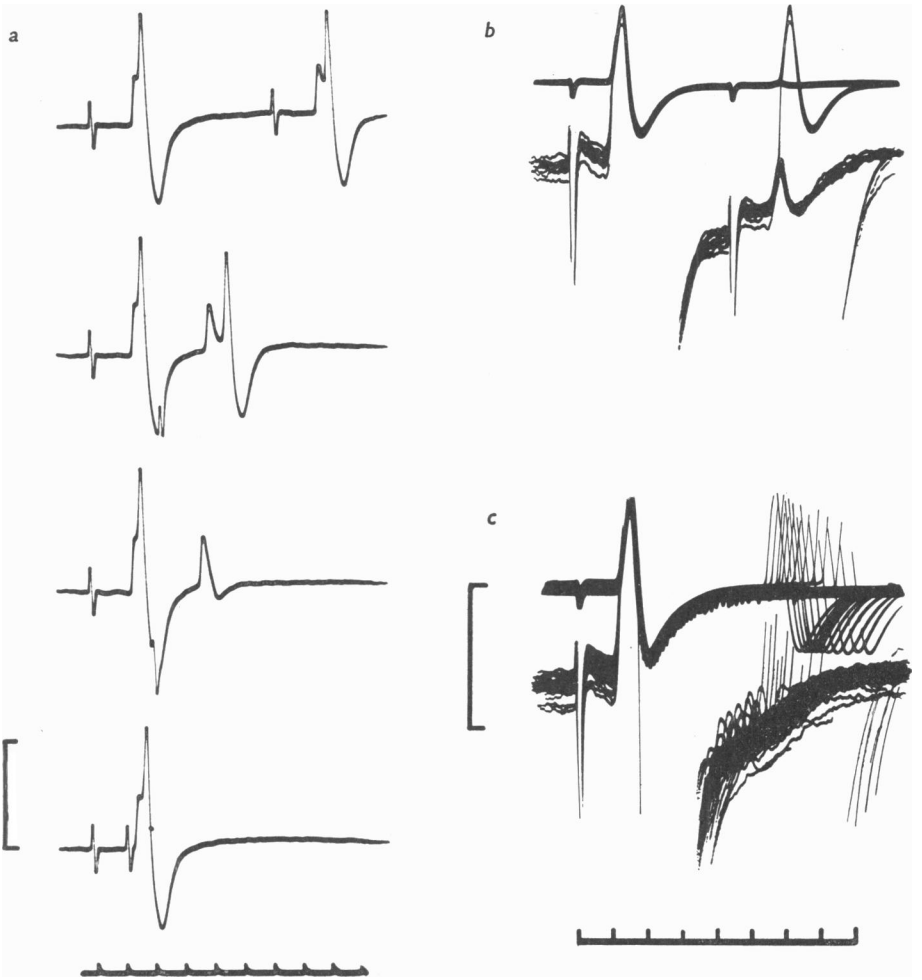
This typical response resembles the 'giant' extracellular spikes which have been reported in cerebellar Purkinje cells (Granit & Phillips, 1956), Betz cells (Phillips, 1959), lateral geniculate neurones (Freygang, 1958), and many other neurones of the central nervous system. In the present investigation 62 units with stable spike amplitudes ranging from 5 to 73 mV peak-to-peak have been seen; 28 of these were over 20 mV. All were found at or near the layer of mitral cell bodies, with brief unvarying latencies between 0·9 and 2·0 msec. These spike potentials are therefore considered to register the invasion of mitral cells by antidromic impulses.

The stability and duration of contact, and the low level of resting activity (less than 10 impulses/sec in all units studied), indicated that injury was not appreciable. Most of the giant spikes survived for 30–60 min and in several cases contact was maintained for periods up to 3½ hr with no apparent sign of injury. Entrance into the cell was marked by a shift of the d.c. potential level to 20–60 mV negative, attended by a short-lived injury discharge or merely silence. These features are all common findings in similar studies (e.g. cerebellar Purkinje cells (Granit & Phillips, 1956)). Spikes with persistent monophasic positive or negative potentials occurred rarely, deteriorated rapidly, and are excluded from this study. Slow 'dendritic' spikes of long duration (Tasaki, Polley & Orrego, 1954) were never encountered.

The generation of antidromic impulses

In Text-fig. 3*a*, upper record, the first spike has an inflexion on the positive-going phase. This is accentuated in the response to a testing shock introduced shortly after the conditioning spike. As the shock intervals are shortened in successive sweeps (lower records), the two components of the testing spike are separated; the second component then fails, as does the first at the shortest interval. This sequence is patently similar to that for the fragmentation of motoneurone spikes (Brock, Coombs & Eccles, 1953) and Betz cell spikes (Phillips, 1959). These two components presumably

register the successive invasion by the antidromic impulse of two successive regions of excitable membrane in the mitral cell. To avoid assuming the identity of these membranes with specific regions (e.g. initial axon, soma and dendrites), we shall adopt the usage of Fuortes *et al.* (1957), and refer



Text-fig. 3. Units recorded at mitral cell body layer. Paired shocks to lateral olfactory tract. *a*, conditioning and testing shocks both 220 μA (about twice threshold); *b*, another unit; upper trace, low-gain d.c. recording; lower trace, simultaneous high-gain a.c. recording; conditioning and testing shocks both 600 μA (about twice threshold); 10 sweeps superimposed; *c*, same, 30 mins later; both shocks 400 μA (just over threshold); many superimposed sweeps with gradually decreasing shock intervals. Time marker, msec. Voltage calibration 10 mV in *a*; calibration at *c* is 50 mV for low-gain, 3 mV for high-gain, recordings in *b* and *c*. In this and other records positivity of the recording tip is upwards, negativity downwards.

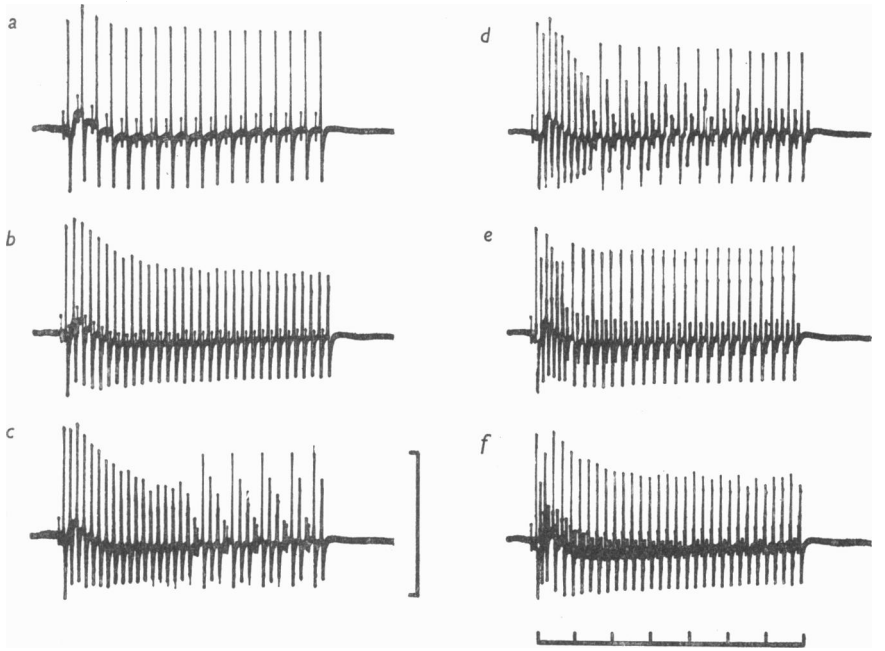
to the successive membranes as the A and B membranes, and to antidromic impulses invading these membranes as the A and B impulses. The A and B impulses are recognizable as the A and B components of the spike potential. Our 'giant' spikes are A–B spikes, and register the invasion of both membranes. When A–B transmission becomes hazardous, the A and B components are separated by inflexions (e.g. Text-fig. 3*a*, second record); and when an A impulse fails altogether to invade the B membrane an isolated A spike is recorded (e.g. Text-fig. 3*a*, third record).

The majority of giant spikes in mitral cells had a smooth positive upstroke, indicating a close coupling between A and B membranes. Such a spike is shown in Text-fig. 3*b*. In the upper sweep, at low-gain amplification, both conditioning and testing spikes (when successful) have smooth conformations. At slightly different intervals, however (not illustrated), partial A–B separation was seen.

The simultaneous high-gain recording in Text-fig. 3*b* discloses a small, monophasic positive spike obtained in isolation when the large spike fails. When the latter is successful, it rises from the peak of the small spike. Text-fig. 3*c*, in which many sweeps are superimposed with decreasing testing intervals, shows the absence of small spikes at intervals less than 2·4 msec, their isolation from 2·4 to 4·2 msec, and the generation of a full A–B spike at longer intervals. A similar small spike was observed in several other units. It was all-or-none and monophasic positive, contrasting with the positive-negative polarity of all other unitary responses found in these experiments, and making it unlikely that it represents a spike in a neighbouring mitral cell. The A spike, when present, always arose from the summit of the small spike, indicating that the small spike registers activity just preceding the activation of the A membrane. It thus appears analogous to the 'M' spike recorded intracellularly in motoneurones (Brock *et al.* 1953) and Betz cells (Phillips, 1959), where it was attributed to the antidromic impulse in the myelinated axon.

Antidromic impulse generation was also investigated with repetitive stimulation of the lateral olfactory tract. The giant spike in Text-fig. 4 follows each volley at 125 c/s in *a* (note shock artifacts). At 225 c/s (*b*) there is a marked decrease in amplitude of successive spikes. At 260 c/s, during the second half of the train, the antidromic impulses sometimes fail to invade the B membrane (Text-fig. 4*c*); after each such failure (registered by an isolated A spike) the amplitude of the next A–B spike recovers considerably, but the ensuing spikes grow smaller until A–B transmission fails again. At 300 c/s A–B transmission is still more hazardous (Text-fig. 4*d*); at still higher rates antidromic invasion of the A membrane also becomes impaired, as revealed by decreasing amplitude and occasional absence of the A spikes (Text-fig. 4*f*).

These results are similar to those reported in motoneurones (Brock *et al.* 1953), and the process of impulse fragmentation appears to be essentially similar to that found in paired shock experiments (cf. Text-fig. 3*a*). A striking feature is the stepwise reduction in B spike amplitude. Paired shock studies also demonstrated partial responses of B membrane, similar to those seen in motoneurones by Fuortes *et al.* (1957) and by Coombs, Curtis & Eccles (1957).



Text-fig. 4. Unit recorded at mitral cell body layer. Repetitive stimulation of lateral olfactory tract. Shocks 400 μA (threshold 310 μA). Increasing stimulation frequency from *a* to *f* of 125, 225, 260, 295, 350 and 420 c/s, respectively. Time marker, 20 msec divisions; voltage calibration 10 mV.

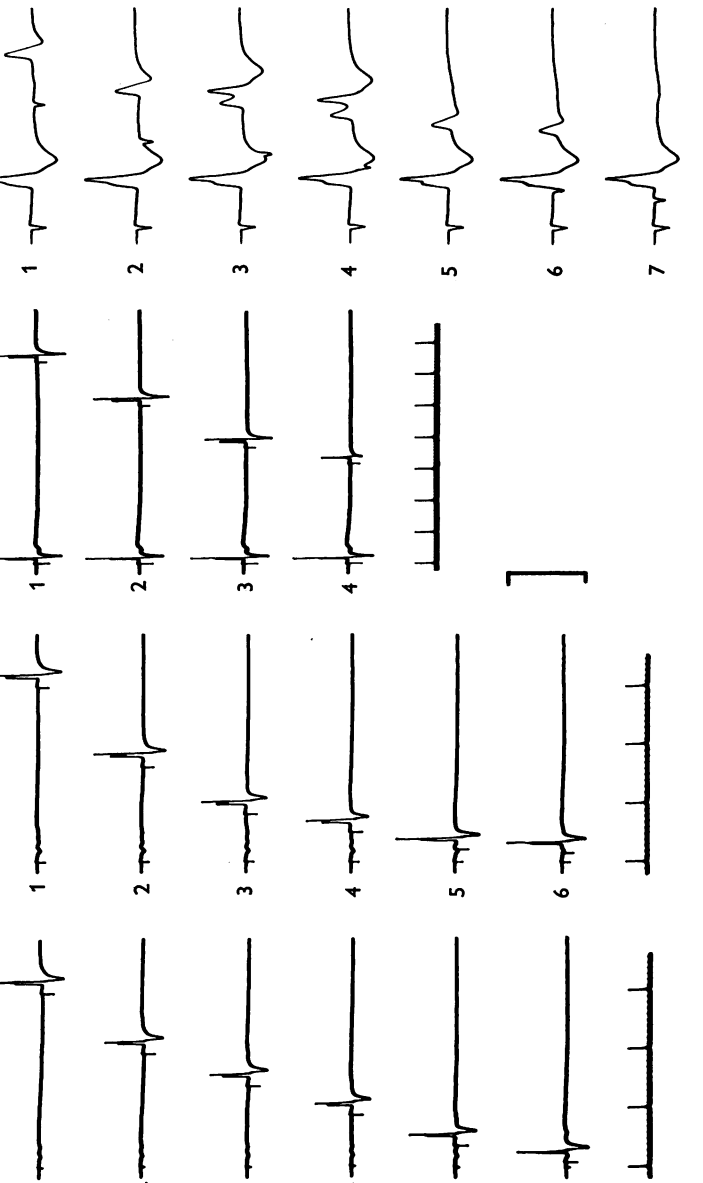
Text-figure 4 illustrates that the ease with which A–B impulse transmission could be made to fail did not necessarily depend upon an A–B inflexion in the unconditioned response. On the other hand, there was often no A–B separation at the critical intervals or frequencies for complete spike failure (cf. Text-fig. 3*b* and *c*). Isolated A spikes in response to single tract shocks were never seen. These features contrast with those obtained from intracellular studies of motoneurones, in which isolated A impulses frequently occur, and B impulses often fail at low rates of repetitive antidromic stimulation while A impulses continue (Brock *et al.* 1953). The safety factor for A–B transmission therefore appears to be higher in mitral cells than in motoneurones.

The ability of giant spikes to follow high rates of lateral olfactory tract stimulation supports their identification as antidromic impulses in mitral cells. Thus, in the majority of units identified as mitral cells by other criteria, B spikes followed frequencies of 150–250 c/s, and A spikes sometimes continued to follow up to 500 c/s (cf. Text-fig. 4). In many cases, however, the A–B spike failed completely at much lower rates, occasionally as low at 2 c/s. A similar situation obtained in the paired shock experiments. In 40 units identified as mitral cells blockage of a second antidromic impulse usually occurred at relatively short testing intervals (1·9–7·6 msec), as in Text-fig. 3. In eight cases, however, complete blockage of a testing spike response occurred over much longer intervals (24–170 msec). The duration of these intervals was often related to the strength of conditioning shocks, as will be described in the next section.

Long-term changes in mitral cell excitability

The early blockage of antidromic invasion of the B membrane, seen in Text-fig. 3, was presumably due to refractoriness of the B membrane in the wake of the preceding impulse. Any later impairment of A–B transmission, on the other hand, might be due to synaptic inhibition of the mitral cell, brought about (directly or through interneurones) by impulses in the other axons that were inevitably stimulated in the lateral olfactory tract. Clear evidence of the effects of impulses in other axons in the tract has been obtained by grading the strength of conditioning shocks to the tract. When these are below threshold for the axon of the mitral cell sampled by the recording micro-electrode, any subsequent depression of A–B transmission in that cell (revealed by a testing shock strong enough to excite its axon) cannot be due to refractoriness of the B membrane. It must, therefore, be due to some change impressed on that membrane by impulses in the 'extraneous' axons. These axons may be those of other mitral cells, and the effect on the sampled mitral cell may be due to impulses in the recurrent axon collaterals of its neighbours in the bulb. We cannot, however, exclude the possibility that the effect may be due, in part, to stimulation of centrifugal axons in the lateral olfactory tract.

Such an experiment is illustrated in Text-fig. 5. Every record begins with the artifact of the conditioning shock to the lateral olfactory tract, with the antidromic response recorded from the layer of mitral cell bodies. For the records of series *a* and *b* the shocks were subliminal for the axon of the sampled mitral cell; small waves only are recorded, and there are no later deflexions (see records *a* 1 and *b* 1, which serve as controls). A second (testing) tract shock of fixed strength, supraliminal for the sampled mitral axon, reveals the state of the B membrane at different times after the first antidromic volley. The intervals are shortened from above downwards



Text-fig. 5. Unit recorded at mitral cell body layer. Paired shocks to lateral olfactory tract. *a*, conditioning shocks 490 μA; testing shocks 1·6 mA; threshold 1·0 mA; *b*, conditioning shocks 880 μA; *c*, conditioning shocks 1·6 mA; *d*, same as *c*, faster sweeps. In all series shock interval decreases from upper to lower records. Time marker for *a*–*c*, in 10 msec divisions; for *d* msec; voltage calibration 25 mV. Controls: the response to the conditioning shocks can be seen at the beginning of the uppermost records of *a*, *b*, *c* and *d*. The giant 'spikes' evoked by the conditioning shocks in *c* and *d* serve as controls of (1) spike amplitude and (2) the normal A–B inflexion for this cell.

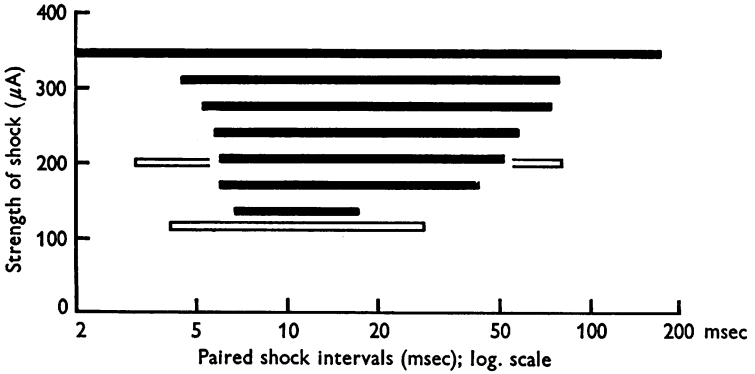
in every series. Control records of the normal state of the B membrane (shape of A–B inflexion, amplitude of B component of spike) are provided at the beginning of records *c* 1–4 and *d* 1–7, in which the conditioning shocks were supraliminal for the axon of this mitral cell.

In series *a* impulses in 'extraneous' tract axons caused a hesitation in A–B transmission, revealed by the exaggerated A–B inflexion, and also diminished the amplitude of the B component of the giant spike. The effect was definite at 8·6 msec (record *a* 4); some effect was still present at 30 msec (record *a* 1). These actions were intensified when the size of the conditioning antidromic volley was increased for series *b*. The time of onset was shortened from 8·6 to 5·2 msec (compare *b* 4 with *a* 4). Further increase in size of the conditioning volley for series *c*, to include the axon of this mitral cell, caused a total failure of A–B transmission at 32 msec (*c* 4), and lengthened the period of depression of B membrane to more than 65 msec (*c* 1).

These effects can be distinguished experimentally from the earlier refractoriness of the B membrane. Record *d* 7, for which the shock interval was 0·9 msec, shows complete refractoriness. As the interval was lengthened, the A membrane first recovered (*d* 6, *d* 5) and then the B membrane (*d* 4, *d* 3). At about 3·2 msec interval the B impulse again failed (*d* 2), and was not restored until the interval was lengthened to about 37 msec (record *c* 3).

The impairment of A–B transmission and B spike amplitude in series *a* and *b* is clearly due to activity over pathways which do not include the axon of this mitral cell. The decrease in the latency of onset, and increase in duration and intensity, of these effects with stronger conditioning shocks are characteristic of mediation over a synaptic pathway. The recovery of A–B transmission at short intervals in *d* is similar to that demonstrated in motoneurones by Brock *et al.* (1953; see their Fig. 8, p. 442), who attributed it to 'the combined effects of partial recovery from relative refractoriness and the relative supernormality associated with the negative after-potential'. It is likely that recovery in *d* occurs in the interval between absolute refractoriness and the arrival of activity in the 'extraneous' pathway. The onset, duration and intensity of this activity are related to the strength of conditioning shocks, in other words to the number of 'extraneous' axons involved.

The duration of the periods of blockage of antidromic invasion imposed by the 'extraneous' pathways on two other cells are illustrated by the open and filled bars in Text-fig. 6. The relation of blockage to conditioning stimulus strength, subthreshold for the axon of the sampled mitral cell in both cases, is clearly evident. The intervals for blockage or impairment of A–B transmission varied widely in different cells. The interval of onset, following 'subthreshold' conditioning volleys, ranged from 3·0 to 7·4 msec;

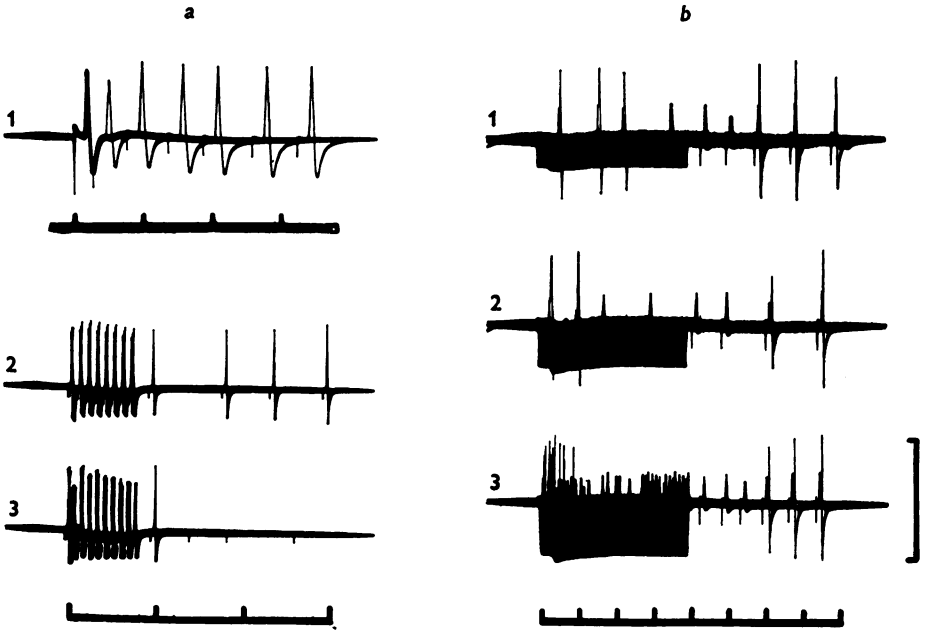


Text-fig. 6. Relation between strength of conditioning shocks to lateral olfactory tract (ordinates) and duration of blockage of testing antidromic impulses in two mitral cells. Open bars: first cell; spikes were recorded from layer of mitral cell bodies. Threshold for axon of this mitral cell was 750 μA. Bars indicate period of blockage of testing antidromic spikes. Filled bars: similar study of another cell recorded at mitral cell body layer. Threshold for this cell's axon was 330 μA.

the interval for recovery ranged from 18 to 80 msec. It was notable that neighbouring cells usually varied widely in these intervals.

A single antidromic volley did not always succeed in evoking these long-lasting depressions. In such cases repetitive volleys were sometimes effective. Thus Text-fig. 7, record *a* 1, shows that a single conditioning antidromic volley, large enough to include the axon of this mitral cell, had no delayed depressing action on the testing antidromic impulses. In these records, which are formed from many superimposed sweeps, the control response to the conditioning tract shock appears as a thickened tracing; on this, the testing antidromic impulses and their shock artifacts appear as thinner lines. Record *a* 2 shows that eight conditioning volleys (smaller but still large enough to include this mitral axon) were also ineffective at 260 c/s. In record *a* 3 the stimulus frequency was increased to 530 c/s without changing the strength. The 'control record' shows that this mitral cell responded to only half the tract shocks. A testing antidromic impulse succeeded at about 50 msec after the onset of the conditioning stimulation, but three later testing shocks failed to produce antidromic impulses. The fact that the number of antidromic impulses actually evoked in this unit by the conditioning stimuli was similar in *a* 2 (8 impulses) and *a* 3 (10 impulses) proves that the antidromic blockage does not depend on a conditioning effect of antidromic A–B impulses, but on the rapid repetitive activation of 'extraneous' axons; the effect on this unit has a long latency of onset ($>$ 70 msec from the beginning of the stimulation) and a long duration ($>$ 150 msec).

A shorter latency of onset and shorter duration of blockage is shown in another mitral cell in Text-fig. 7*b*. The frequency of the repetitive conditioning stimuli was kept at 540 c/s. For record *b* 1 these stimuli were below threshold for the cell's axon. Supraliminal testing shocks showed that antidromic A–B transmission was impaired towards the end of the conditioning stimulation, as shown first by increased A–B delay, then later by



Text-fig. 7. Units recorded at mitral cell body layer (original records traced for reproduction as a line drawing). *a* 1, paired shocks to lateral olfactory tract; conditioning shocks 550 μA; testing shocks 190 μA; threshold 140 μA; superimposed sweeps with decreasing shock intervals. Time marker, 50 msec divisions. *a* 2, conditioning tetani and single testing shocks, both 170 μA; tetanus frequency 260 c/s. *a* 3, tetanus frequency 530 c/s. Time marker, 50 msec divisions. *b*, another unit; conditioning tetani 400, 580 and 800 μA in records *b* 1, *b* 2 and *b* 3 respectively; tetanus frequency 540 c/s. Single testing shocks 930 μA; test axon threshold 800 μA. Superimposed sweeps with decreasing shock intervals. Time marker, 10 msec divisions; voltage calibration, 50 mV for *a* 2 and *a* 3 records, 25 mV for other records.

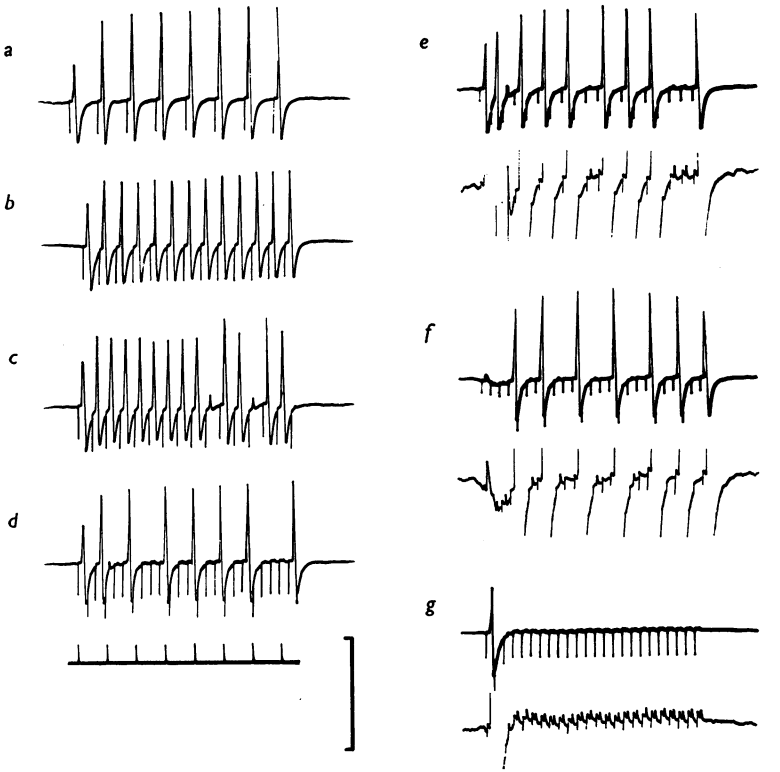
isolated A spikes; A–B transmission recovered about 60 msec from the onset of stimulation. For record *b* 2 the conditioning stimuli were strengthened, but were still subliminal for the test axon: the onset of impaired transmission was earlier. The period of impairment was not further lengthened by making the conditioning shocks just supraliminal for the test axon (record *b* 3): the onset was not advanced, and recovery still occurred about 60 msec from the onset of stimulation.

If the changes in excitability are due to an effect of 'extraneous' impulses on the membrane potential or membrane conductance of the mitral cell, systematic changes in the amplitude of the giant spikes might be expected during the course of the effect (see Discussion). Text-figure 8 illustrates such changes. The giant spike evoked in this cell by single tract shocks was over 50 mV in amplitude before repetitive stimulation was begun (Text-fig. 3*c*). After some bursts of repetitive stimulation the amplitude of the first spike of each of the trains in Text-fig. 8, records *a–e* and *g*, became smaller than the control spike of Text-fig. 3*c*. Unfortunately the cell died before a final control of spike amplitude could be recorded after a period of rest: we cannot, therefore, be sure that the decline in amplitude was reversible, and due, as we suspect, to a cumulative depolarizing effect of several brief trains of repetitive stimulation. But, however produced, this presumed depolarization provided the necessary background for the experiment of Text-fig. 8. Record *a* shows that the spike amplitude was not merely restored, but grew to exceed its original value (cf. Text-fig. 3*c*) when the lateral olfactory tract was stimulated at 100/sec with shocks 2·5 times threshold for this cell's axon.

The effect was not due to the invasion of the B membrane by the first antidromic impulse (i.e. to a hyperpolarizing after-potential). Record *f* shows a similar effect when the first impulse invaded the A membrane only. (For records *e* and *f* the shocks were weakened to near-threshold for the test axon; the frequency of stimulation was raised, but the antidromic A–B impulses of this cell did not follow it, their frequency remaining comparable to that of record *a*.)

When shocks of the strength used for record *a* were repeated at higher frequencies, the effects became complex. The spikes of record *b* (170/sec) are smaller than those of record *a*. In record *c* (200/sec) two instances of antidromic A–B blockage are seen. After each of these failures of the impulse to invade the B membrane the amplitude of the ensuing spike became almost equal to that of the spikes in record *a*. In record *d* (320/sec) the A membrane failed to follow; eight full A–B impulses were generated during this train (the same number as were fired in record *a*), their spike amplitudes being comparable to those in *a*. Only one A–B impulse was set up by a similar stimulus train in record *g*. The simultaneous high-gain record shows that the 'M' impulse (cf. Text-fig. 3*b*, *c*) responded to every shock. Thus there is no question of a failure of response of the axon at the site of stimulation. The probable meaning of these results will be considered in the Discussion.

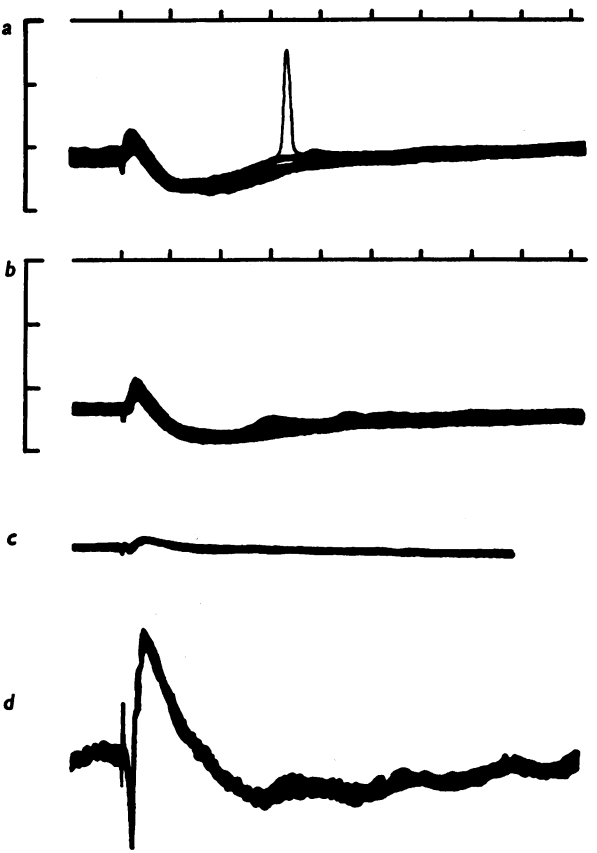
Direct evidence for the relation of these long-term changes in excitability to changes in membrane potential is shown in Text-fig. 9. This unit was encountered at the mitral cell body layer as an extracellular spike



Text-fig. 8. Same unit as in Text-fig. 3 *b* and *c*; repetitive shocks to lateral olfactory tract. *a–d*, shocks strengths 750 μA; axon threshold 300 μA; increasing tetanus frequencies of 100, 170, 200 and 320 c/s. *e–g*, upper sweeps, low-gain recording; lower sweeps, simultaneous high-gain a.c. recording. *e*, shock strengths 370 μA; tetanus frequency 250 c/s, raised to 320 c/s in *f* and *g*. Shocks in *g* strengthened to 750 μA. Time marker, 10 msec divisions; voltage calibration, 6 mV for high-gain recordings, 50 mV for other recordings.

responding with a short, fixed latency to lateral olfactory tract stimulation, and was thereby identified as a mitral cell. Paired shocks disclosed a long-lasting blockage of testing responses. There was then an abrupt shift of the resting d.c. potential level to 50 mV negative in *a*, increasing to 56 mV in *b*. Despite the cessation of spike responses (one injury discharge is seen in *a*), there is an initial small depolarization followed by a long-lasting hyperpolarization of the cell membrane, which has an onset at 4–5 msec, a duration of 30–60 msec, and a maximal amplitude of 11–12 mV. At the end of the tracing in *a* the membrane potential is 2 mV more depolarized than the resting value; in *b* it is still 3 mV hyperpolarized. After deterioration of the membrane potential level, control recordings in *c* and *d* show the typical extracellular potential wave response.

The long-term suppression of mitral cell excitability was sometimes produced by very weak conditioning shocks to the lateral olfactory tract, as in Text-fig. 6. It was also found, as in Text-fig. 8, after removal of the contralateral bulb, anterior commissure and most of the forebrain, except for the immediate region of the lateral olfactory tract and a small area overlying the anterior cerebral vasculature which supplies the tract and bulb. These controls rule out the participation of the contralateral bulb or the forebrain in the extraneous pathways affecting mitral cell excitability



Text-fig. 9. Unit recorded at mitral cell body layer (original records traced for reproduction as a line drawing). *a*, single shocks to lateral olfactory tract at 350 μA (just over threshold for extracellular spike response). Time scale in 10 msec divisions also represents extracellular d.c. potential level; voltage scale at left, in 25 mV divisions, indicates negative displacement of d.c. potential level. 10 sweeps superimposed. *b*, same, recorded shortly after *a*. *c*, micropipette withdrawn slightly; d.c. potential level returned to base line; extracellular summed wave response at same amplification and d.c. recording. *d*, same at higher gain and a.c. recording.

in these experiments. They make it unlikely that stimulus spread to the rostral limb of the anterior commissure was significantly involved. Although possible excitation of any centrifugal fibres in or near the lateral olfactory tract could not be controlled by this means, identical suppression of mitral cell excitability is produced by shocks to the olfactory nerves (Shepherd, 1963*a*) and to the olfactory mucosa (Yamamoto & Iwama, 1962), which obviate the excitation of extrabulbar centrifugal fibres. The results are also similar to those obtained in hippocampal pyramidal cells (Kandel *et al.* 1961) by stimulation of the de-afferented fornix. It would appear that intrabulbar neuronal systems, activated by recurrent collaterals of the mitral axons, account for most, if not all, of the observed changes in mitral cell excitability. We emphasize, however, that we cannot exclude the possibility that these intrabulbar neuronal systems are also activated by centrifugal fibres running in the lateral olfactory tract.

DISCUSSION

Antidromic impulses in mitral cells

Mitral cells have been identified by the criteria of antidromic impulse invasion and of localization of the recording tip to the layer of mitral cell bodies. The amplitudes of extracellularly-recorded 'giant' spikes have reached 73 mV. The conditions under which such 'giant' spikes are recorded have been considered by Granit & Phillips (1956), Freygang & Frank (1959), Eccles (1960), and Rall (1962), and our results have thrown no fresh light upon these conditions. Further study of mitral cell giant spikes is in progress. That large amplitudes could be obtained, that contact could be maintained for up to 3½ hr under stable conditions, and that resting activity was minimal or absent, argue against appreciable injury to the cells (cf. Granit & Phillips, 1956).

The stages of antidromic impulse invasion in mitral cells are in agreement with those in motoneurones (Brock *et al.* 1953), Betz cells (Phillips, 1959), hippocampal pyramidal cells (Kandel *et al.* 1961) and other neurones. The discussion by Brock *et al.* (1953) of the role of refractoriness in determining the successive blockage of a testing spike following a conditioning spike adequately accounts for the results in Text-figs. 3 and 4. Our results demonstrate the utility of 'giant' extracellular spikes in elucidating the properties of antidromic invasion when intracellular recordings are hard to obtain. Thus, the small spike in Text-fig. 3*b* and *c* and Text-fig. 8 *e–g* may be taken to register the antidromic impulse in the myelinated axon, as in motoneurones (Brock *et al.* 1953) and Betz cells (Phillips, 1959). The monophasic positive conformation of this spike also suggests activity at a distance from the recording site, which is presumably

on somatic membrane. Partial responses of B membrane, as in Text-fig. 4 and other cells in the present study, have been fully discussed for motoneurones by Fuortes *et al.* (1957) and Coombs *et al.* (1957) in terms of partial depolarization of refractory B membrane. If the stepwise amplitudes of these partial responses in Text-fig. 4 correspond to areas of activated membrane, the latter might be determined by geometrical dispositions of somatic or dendritic membrane or by the distribution of synaptic or glial contacts (cf. Eccles, 1957).

The present results do not permit a precise identification of the membranes which generate A and B spikes during antidromic invasion of mitral cells. Studies of both peripheral and central neurones suggest that the extent of invasion is variable, and may depend on such factors as synaptic or glial contacts with the cell membrane (Eccles, 1957; Frank & Fuortes, 1961). The uniformity in the conformation and properties of the extracellular spikes in different neurones contrasts with the variability in dendritic structures. In mitral cells, with their long primary dendritic trunks, it was notable that slow 'dendritic' impulses of long duration (Tasaki *et al.* 1954) were never encountered.

Excitability changes in mitral cells

The refractory periods of motoneurones, and particularly of the B membrane, vary widely (Brock *et al.* 1953), and a similar variation in mitral cell spikes could account for the wide range in intervals of blockage and rates of following in mitral cells. This explanation is excluded, however, when the conditioning stimulus is below threshold for the axon of the cell under study. In such cases subsequent suppression of responses in that cell, as revealed by suprathreshold testing shocks, must be due to activity in 'extraneous' axons. Text-figures 5–8 illustrate the prevalence of this activity in mitral cells, and intracellular recordings in Text-fig. 9 indicate that the suppression of excitability is associated with hyperpolarization of the mitral cell membrane. Its onset and duration are closely similar to those demonstrated in Betz cells (Phillips, 1959, 1961), and hippocampal pyramidal cells (Kandel *et al.* 1961) under similar stimulus conditions. In agreement with these workers, and with studies of motoneurones (Eccles, 1957), it may be termed an inhibitory post-synaptic potential (IPSP).

Yamamoto & Iwama (1962) have also reported a long-lasting hyperpolarization in mitral cells, though in their records it is preceded by the antidromic spike response. They concluded that, when hyperpolarization was absent, the recording tip was inside the mitral cell axon; but the small diameter of mitral axons (less than $1{\cdot}6\,\mu$ according to Allison, 1953) makes this doubtful. It is more probable that the occasional absence of an IPSP

is related to the absence of any detectable long-lasting suppression of excitability in many of our mitral cells (see Text-figs. 3 and 4). This may be taken to reflect the variation in 'extraneous' activity playing on different mitral cells, but it could possibly be a consequence of injury to the cell by the micropipette tip. Both in Text-fig. 9 and in Yamamoto & Iwama's (1962) records the low membrane potentials indicate depolarization from injury. More reliable records are needed, though intracellular recordings from mitral cells, as Yamamoto & Iwama also attest, are extremely difficult to obtain.

In view of this difficulty the possibility that changes in 'giant' spike amplitude reflect corresponding changes in membrane potential or membrane conductance is of interest. Granit & Phillips (1956) pointed out that in Purkinje cells 'even in the absence of intracellular records, it seems safe to conclude...that diminution of the (giant) spike is a sign of cell depolarization'. Conversely, increased amplitude of the extracellularly-recorded giant spike may mean that the membrane polarization has been increased, either by after-polarization following an impulse or by inhibitory synaptic action. The initial smallness of the giant spike of Text-fig. 8 may have been due to a background of membrane depolarization brought about by a cumulative action of repeated bursts of repetitive stimulation, persisting during the intervals between the bursts. Restoration of spike amplitude during the trains of Text-fig. 8*a*, *d*, *e*, and *f* might then have been due to a repolarizing effect of inhibitory synaptic action during the trains, generated by impulses in the recurrent collaterals of the mitral axons that were stimulated in the lateral olfactory tract. When the mitral cell is made to fire at higher frequencies, as in Text-fig. 8*b* and *c*, the lessened growth of the giant spike is presumably due to relative refractoriness of the B membrane. Certainly when, in record *c*, the B membrane failed to fire, the ensuing A–B spike jumped up to its full amplitude.

In Betz cells (Phillips, 1959, 1961) the hyperpolarizing IPSP subsequent to pyramidal tract stimulation was attributed to activation of intracortical interneurones via recurrent collaterals from neighbouring Betz cell axons, in analogy with 'Renshaw inhibition' in the spinal cord (Eccles, Fatt & Koketsu, 1954). Yamamoto & Iwama (1962) inferred that in mitral cells the IPSP is mediated by a direct connexion between recurrent axon collaterals and secondary dendrites. Kandel *et al.* (1961) reached a similar conclusion for hippocampal pyramidal cells. However, in our experiments the long latency of onset (from a minimum of about 3 msec to over 50 msec) suggests that suppression of mitral cells is mediated by synaptic pathways analogous to those postulated for Betz cells. Recordings have been made from presumed granule cells and tufted cells, and their participation in the control of mitral cell excitability is considered in

a subsequent paper (Shepherd, 1963*b*). Sustained activity in these pathways would account for the long duration of the observed effects. In some pathways the duration of this activity may be self-limited, regardless of the intensity of stimulation, as in Text-fig. 7*b*. The possibility that such activity might be evoked by centrifugal axons, as well as by collaterals of the mitral axons, must always be kept in mind.

It has been noted that the safety factor for antidromic A–B transmission in mitral cells is higher than that in motoneurones. Phillips (1959) reached a similar conclusion for Betz cells, and isolated A impulses are apparently not frequent in hippocampal pyramidal cells (Kandel *et al.* 1961). This may be a common feature of these cells, related possibly to geometrical factors, as Phillips suggests. Despite this higher safety factor, full antidromic spikes in mitral cells did not follow repetitive stimulation above 300 c/s, which contrasts with rates of up to 1320 c/s in other cells (Gordon & Seed, 1961). This could be a consequence of the relatively small diameter of mitral cell axons, but at low frequencies, down to 2 c/s, the suppression of responses sometimes observed must be due, at least in part, to activity in the extraneous pathways. This activity was also shown to account for the long intervals of blockage after suprathreshold conditioning responses (as in Text-fig. 5*c* and *d*). The variations in these properties in neighbouring mitral cells may in part reflect corresponding variations in the intrabulbar pathways acting on mitral cells. The relation of these properties to the specific responses of mitral cells to different odours, and to different odour intensities (Adrian, 1958), is a matter for future research.

SUMMARY

1. In anaesthetized rabbits a correlation has been obtained between the potential waves evoked in the olfactory bulb by lateral olfactory tract volleys and the position of the tip of a recording micro-electrode in relation to the histological laminae of the bulb.

2. Mitral cells in the olfactory bulb have been identified by their antidromic responses to shocks to the lateral olfactory tract, and by the localization of these responses at or near to the layer of mitral cell bodies.

3. 'Giant' spikes, of up to 73 mV peak-to-peak potential, have been regularly obtained in recordings with extracellular micropipettes.

4. Paired and repetitive shocks to the lateral olfactory tract have shown that an antidromic impulse invades successive regions of excitable membrane in the mitral cell. In this physiological property the mitral cells resemble spinal motoneurones and other neurones of the central nervous system, in spite of their different morphology.

5. Conditioning lateral olfactory tract volleys, too weak to excite the axon of a sampled mitral cell, often produce a long-lasting blockage of antidromic invasion following suprathreshold testing shocks. Intracellular recordings indicate that blockage is associated with an IPSP across the mitral cell membrane. By analogy with motoneurones and 'cortical' pyramidal cells, it is suggested that this blockage is mediated by intrabulbar neuronal systems activated by recurrent collaterals of the mitral axons.

6. We have not been able to exclude the possibility that centrifugal fibres running in the lateral olfactory tract were a complicating factor in our experiments.

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EXPLANATION OF PLATES

PLATE 1

A: dorsal and lateral views of the rabbit's brain, showing insertions of recording *a* and guide electrodes *b* and *c* in the olfactory bulb before fixation with formol-saline.

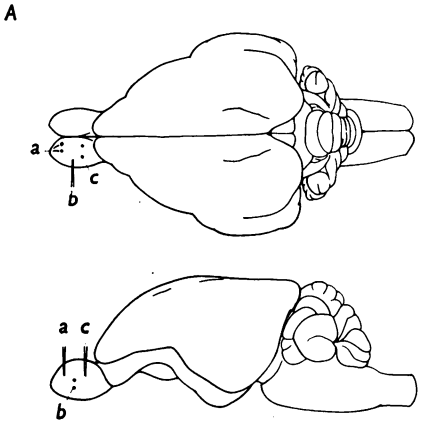
B: correlation of potential wave responses to lateral olfactory tract volleys with histological laminae of the rabbit's olfactory bulb. Thionin-stained section, prepared as described in Methods. The recording track appears large because the pipette was thrust deep into the bulb and left *in situ* until after fixation was complete. Depth scale at left, in 0·1 mm divisions. Potential wave responses at right; 10 sweeps superimposed in each record. Positivity of the recording tip is upwards, negativity downwards. Time marker, 10 msec divisions.

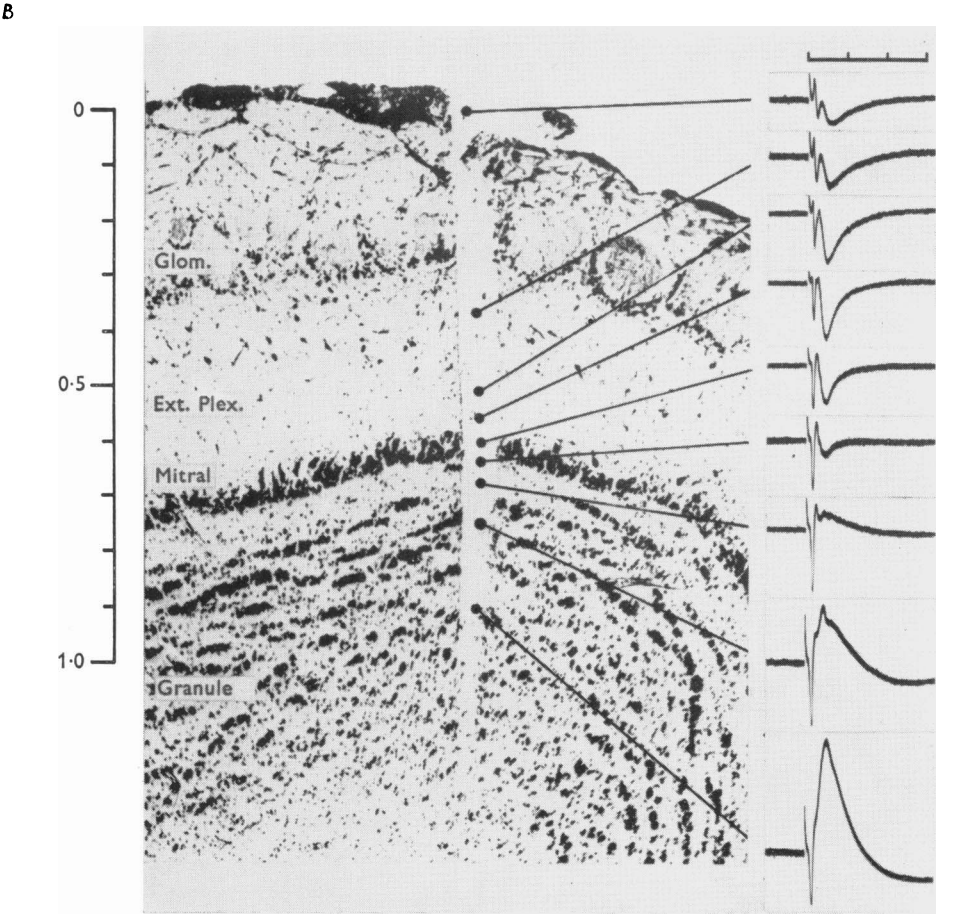
PLATE 2

Thionin-stained section of the olfactory bulb, containing track of recording pipette. Potential wave responses to lateral olfactory tract volleys during this penetration are on the right. Time marker, msec.

PLATE 3

Thionin-stained sections of the olfactory bulb, showing successive sites of a recording track in adjoining sections (*A–D*) of the olfactory bulb. Potential wave responses to lateral olfactory tract volleys during this penetration are on the left. Time marker, msec.





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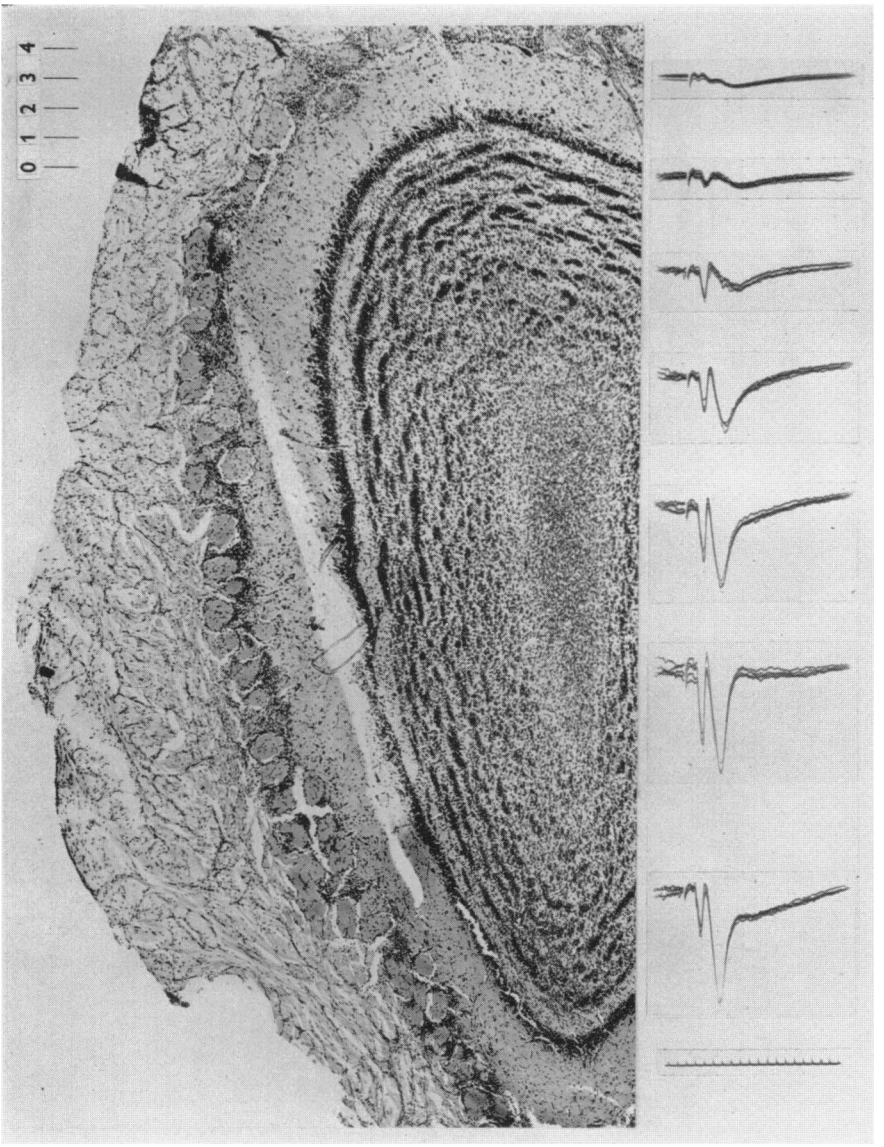

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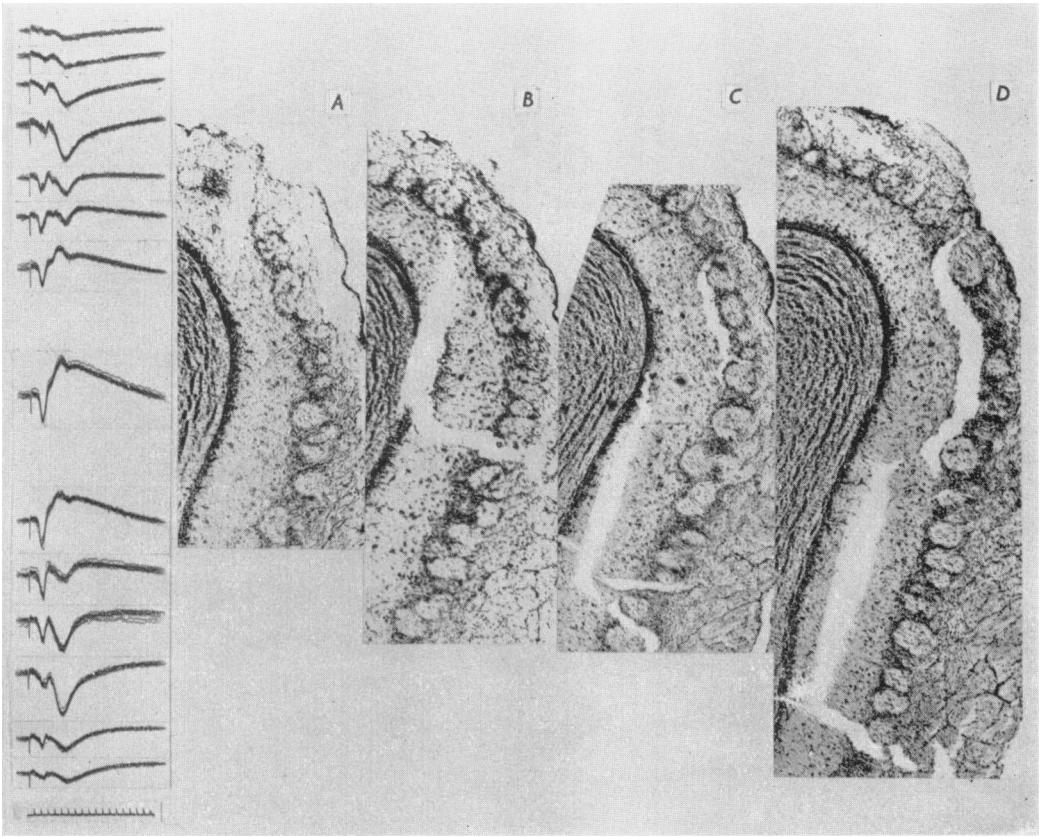
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