J. Physiol. (1978), **279**, *pp.* 569–588
With 9 text-figures
Printed in Great Britain

AN INTRACELLULAR STUDY OF DENDRODENDRITIC INHIBITORY SYNAPSES ON MITRAL CELLS IN THE RABBIT OLFACTORY BULB

BY KENSAKU MORI AND SADAYUKI F. TAKAGI

From the Department of Physiology, School of Medicine, Gunma University, Maebashi, Gunma, 371, Japan

(*Received 30 November 1977*)

SUMMARY

1. In the rabbit olfactory bulb, intracellular potentials were recorded from mitral cells and from neurones in the granule cell layer (g.c.l.) following lateral olfactory tract (l.o.t.) stimulation.

2. Most recordings from mitral cells showed large (5–21 mV) and prolonged (60–650 msec) i.p.s.p.s subsequent to the antidromic spikes. These i.p.s.p.s decreased in amplitude and then reversed in polarity by progressive increase in hyperpolarizing current applied intracellularly. They were accompanied by a prominent and long lasting (up to 100 msec) conductance increase of the mitral cell membrane.

3. Reversed i.p.s.p.s of mitral cells having quite different time courses from the original hyperpolarizing i.p.s.p.s suggest that the inhibitory synapses are widely distributed on the soma and dendrites.

4. E.p.s.p.s could be recorded from g.c.l. cells whose onset latency was approximately 0·6 msec shorter than that of mitral cell i.p.s.p.s. Comparison of the behaviour of e.p.s.p.s in g.c.l. cells and that of mitral cell i.p.s.p. under various conditions of l.o.t. stimulation suggests that these g.c.l. cells are the inhibitory interneurones mediating mitral cell inhibition.

5. The results support the hypothesis of dendrodendritic pathways for activation of granule cells and subsequent inhibition of mitral cells.

INTRODUCTION

Antidromic stimulation of the lateral olfactory tract (l.o.t.) produces a prolonged inhibition of mitral cell activity in the rabbit olfactory bulb (Green, Mancia & Baumgarten, 1962; Ochi, 1963; Phillips, Powell & Shepherd, 1963; Nicoll, 1969). Long lasting i.p.s.p.s have been demonstrated with intracellular recordings from mitral cells (Yamamoto, Yamamoto & Iwama, 1963; Phillips *et al.* 1963; Nicoll, 1969; Reese & Shepherd, 1972). Green *et al.* (1962) suggested that the axon collaterals of mitral cells had a direct inhibitory effect on the mitral cells. However, later physiological studies strongly supported the idea that an inhibitory interneurone, most probably a granule cell, is interpolated in the inhibitory pathway onto the mitral cell (Yamamoto *et al.* 1963; Phillips *et al.* 1963; Shepherd, 1963; Nicoll, 1969). Furthermore, a dendrodendritic pathway for synaptic excitation of granule cells through mitral cell dendrites has been postulated based on the theoretical

analysis of field potentials in the olfactory bulb following l.o.t. stimulation (Rall, Shepherd, Reese & Brightman, 1966; Rall & Shepherd, 1968; for a review see Shepherd, 1972). Electron microscopic studies of the olfactory bulb have revealed reciprocal synapses between mitral cell dendrites and the peripheral processes of granule cells (Fig. 1*B* and *C*) (Hirata, 1964; Andres, 1965; Rall *et al.* 1966; Price & Powell, 1970*a*; Reese & Shepherd, 1972; Willey, 1973). Moreover, it has been reported that nearly all the synapses found on mitral cell dendrites and somata, except in or around the glomerular region, are reciprocal synapses with the peripheral processes of granule cells (Price & Powell, 1970*b*; Reese & Shepherd, 1972). To date, physiological analysis of the inhibitory mechanisms controlling mitral cell activity has been carried out largely by means of extracellular recordings. It was necessary to obtain intracellular recordings from olfactory bulb neurones in order to analyse directly the properties of the inhibitory mechanism.

The first objective of the present study was to clarify the properties of i.p.s.p.s of mitral cells following l.o.t. stimulation in order to ascertain whether or not they have similar characteristics to the i.p.s.p.s of other central neurones. The second was to determine whether or not the inhibitory synapses are widely distributed on mitral cell dendrites and the third was to test the hypothesis of dendrodendritic pathways for excitation of granule cells and subsequent inhibition of mitral cells.

A preliminary account of part of this work has been reported elsewhere (Mori & Takagi, 1977*a*, *b*).

METHODS

Preparations

Seventy-two albino rabbits weighing between 1·7 and 3·2 kg were anaesthetized by I.P. injection of 20% urethane (1–1·2 g/kg) or a mixture of 20% urethane (1 g/kg) and 1% chloralose (5 mg/kg) and later supplemented by I.V. injection of 10% urethane when needed. Fifteen animals were then immobilized with I.V. Flaxedil (gallamine triethiodide) (10 mg/kg) and artificially ventilated through a tracheal canulla. A venous catheter was inserted into the saphenous vein. The animal was then mounted in a stereotaxic instrument (Narishige SN-3). Drainage of the cerebrospinal fluid at the atlanto-occipital linkage was routinely carried out in order to minimize the pulsation of the brain. Openings were made in the dorsal cranium for introducing stimulating electrodes and in the bone overlying the dorsal surface of the olfactory bulb for inserting the recording electrodes. The exposed surface of the olfactory bulb was covered by a mixture of warmed mineral oil and white vaseline in order to reduce cooling and drying. The rectal temperature was monitored and kept within 35·5 and 39 °C by a heating pad.

Stimulation

For stimulation of the l.o.t., bipolar electrodes of acupuncture needles (amalgam of silver, nickel and iron) insulated except for the tip were stereotaxically inserted into the l.o.t. (Fig. 1*A*). The final position of the stimulating electrode was determined following small adjustments to obtain a low threshold antidromic field potential in the olfactory bulb. In ten experiments, the left eye was enucleated and the lateral side of the cranium overlying the l.o.t. was opened in order to place the stimulating electrode directly on the surface of the l.o.t. under visual control. Single square pulses of 0·1 msec in duration were used for stimulating the l.o.t. Stimulation was usually at a frequency of 0·8/sec. At the end of the experiment, a small electrolytic lesion was made at the site of stimulation which was later checked histologically.

Recording

The electrode placement is shown in Fig. 1*A*. For intracellular recording, micro-pipettes filled with 2 M-KCl, 2 M-K citrate or 2 M-K acetate and having 15–80 MΩ resistance were used.

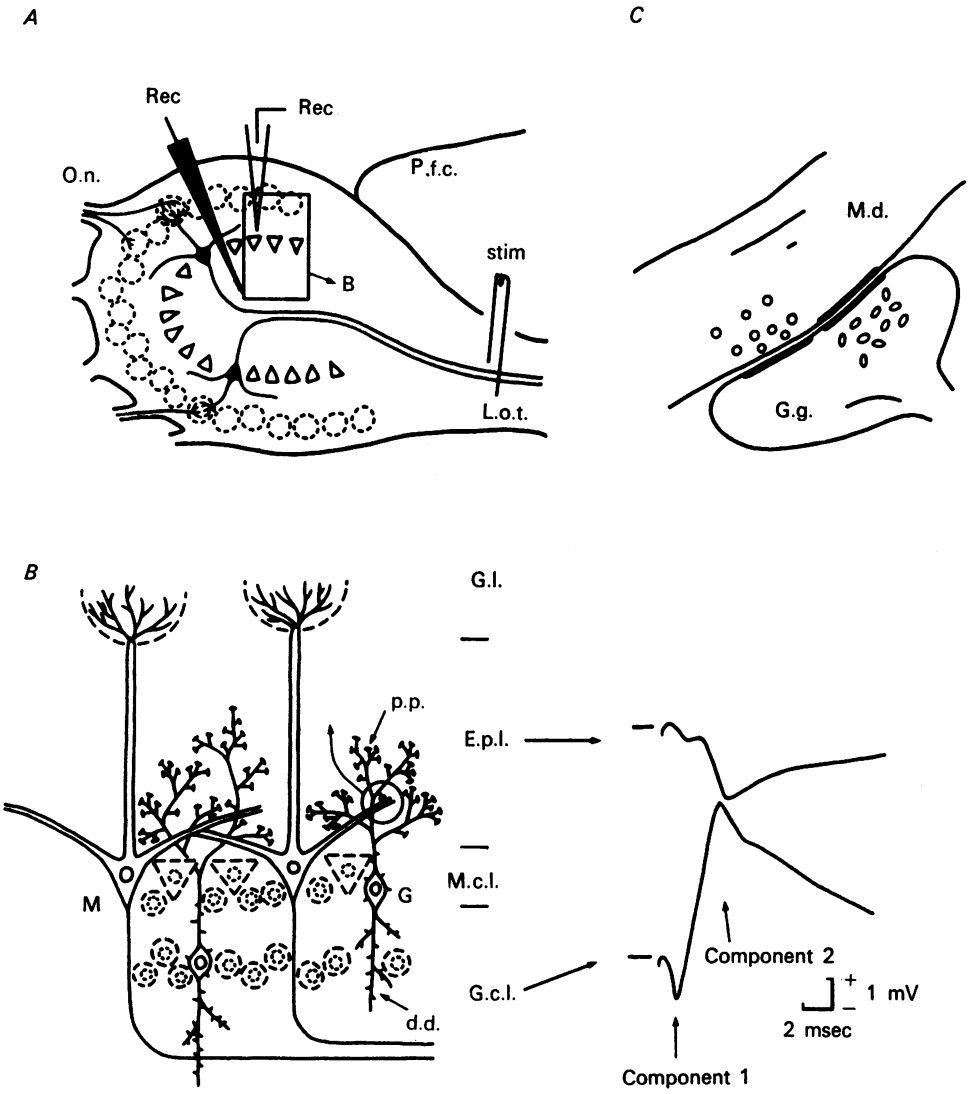

Fig. 1. *A*, diagrammatic illustration of the experimental arrangement showing a stimulating electrode placed in the lateral olfactory tract (L.o.t.) and two types of recording electrodes, a tungsten micro-electrode (black) for recording the field potential in the middle area of the granule cell layer (G.c.l.) and a glass micro-electrode (white) for intracellular recordings. On., olfactory nerve; P.f.c., prefrontal cortex. *B*, the enlarged schematic illustration of the region enclosed in the rectangle in *A* (arrow with letter *B*). Histological layers are shown at right; G.l., glomerular layer; E.p.l., external plexiform layer, M.c.l., mitral cell layer; g.c.l., granule cell layer. Only two types of neurones are shown in this diagram; mitral cells (M) and granule cells (G). P.p., peripheral process of granule cell; d.d., deep dendrite of granule cell. The traces on the right of the diagram are the extracellular field potentials recorded in the e.p.l. (upper trace) and the g.c.l. (lower trace) following l.o.t. stimulation. The latter are composed of first negative wave (component 1) and the following large positive wave (component 2). *C*, enlarged illustration of the region enclosed in the circle in *B* showing dendrodendritic reciprocal synapses between mitral cell dendrite (M.d.) and the gemmule of a granule cell (G.g.).

The micro-pipettes were driven vertically from the dorsal surface of the olfactory bulb by a conventional oil micromanipulator. Contact of the micro-pipette tip with the surface of the olfactory bulb was adjusted under direct vision using a binocular microscope. In addition, a tungsten electrode was inserted in the middle area of the granule cell layer (g.c.l.) in the olfactory bulb for recording the field potential in the g.c.l. The micro-electrode was connected to a pre-amplifier (Nihon-Koden MEZ 9001) designed for both recording and passing currents through single micro-electrodes. The recorded signals were then amplified and displayed on an oscilloscope and photographed on X-ray film. The resting membrane potential was simultaneously monitored by a DC pen recorder. A silver plate coated with silver chloride was placed on the temporal muscle as a reference electrode.

RESULTS

Identification of mitral cells

The micropipette was progressively advanced from the dorsal surface of the olfactory bulb (Fig. 1*A*) and extracellular field potentials evoked by l.o.t. stimulation were recorded. The field potential has a characteristic pattern at each layer in the olfactory bulb so that the position of the micro-pipette can be determined accurately (cf. Phillips *et al.* 1963; Rall & Shepherd, 1968). For example, the second negative wave of the field potential in the external plexiform layer (e.p.l.) reverses its polarity at or near the mitral cell layer and becomes a large positive wave in the g.c.l. (component 2 in Fig. 1*B*). Impaled cells at or near the mitral cell layer were identified as mitral cells when they were activated antidromically from the l.o.t. (cf. Fig. 2*A*(*a*)). The latencies of the antidromic spike potentials of mitral cells varied from 1·0 to 2·1 msec (mean $1{\cdot}7 \pm 0{\cdot}3$ msec S.D., $n = 242$) but the following observations on the properties of l.o.t.-evoked i.p.s.p.s described in this communication were similarly seen in mitral cells with different antidromic activation latencies. The data presented here have been derived from stable neurones which showed no remarkable fluctuation of the resting membrane potential. The level of spontaneous discharges of these mitral cells were usually less than 5 impulses/sec and the resting membrane potentials ranged from -45 to -72 mV. Stable recording times ranged from 4 min to more than 2 hr.

Inhibitory post-synaptic potentials of mitral cells

In almost all (238 out of 240) mitral cell recordings, stimulation of the l.o.t. produced a large and prolonged hyperpolarization in addition to an antidromic spike potential, as shown in Fig. 2*A*(*b*) and (*c*). This hyperpolarization was clearly distinguished from an afterhyperpolarization of the antidromic spike by the following two observations: first, when the stimulus strength was adjusted to just subthreshold for antidromic activation of the cell, the hyperpolarization could be evoked without antidromic spike invasion (Fig. 2*B*); secondly, the hyperpolarization remained even in those cells in which the spike generating mechanism was damaged (Fig. 2*C*). The inhibitory effects of the hyperpolarization could be seen as a depression of spontaneous activity and as an interference with synaptic activation from olfactory nerve input (*cf.* Mori & Takagi, 1975). In addition, intracellular application of hyperpolarizing current reduced the amplitude of the hyperpolarizing potential and eventually converted it into a depolarization (*cf.* Fig. 4). These observations indicate that the hyperpolarization is an inhibitory post-synaptic potential (i.p.s.p.) similar to that found in spinal motoneurones (Coombs, Eccles & Fatt, 1955) and

other nerve cells (*cf.* Eccles, 1964). Fig. 2*C* shows the finely graded nature of the i.p.s.p.s in relation to the intensity of l.o.t. stimulation. As the stimulus strength was increased, both the amplitude and duration of the i.p.s.p.s gradually increased. Supramaximal l.o.t. stimulation (i.e. stimulus strength adjusted so that an increase would not further augment the evoked potentials) produced large and prolonged i.p.s.p.s whose peak amplitudes ranged from 5 to 21 mV (mean 14 ± 4 mV S.D., $n = 173$). However, the amplitudes were influenced by the level of the membrane

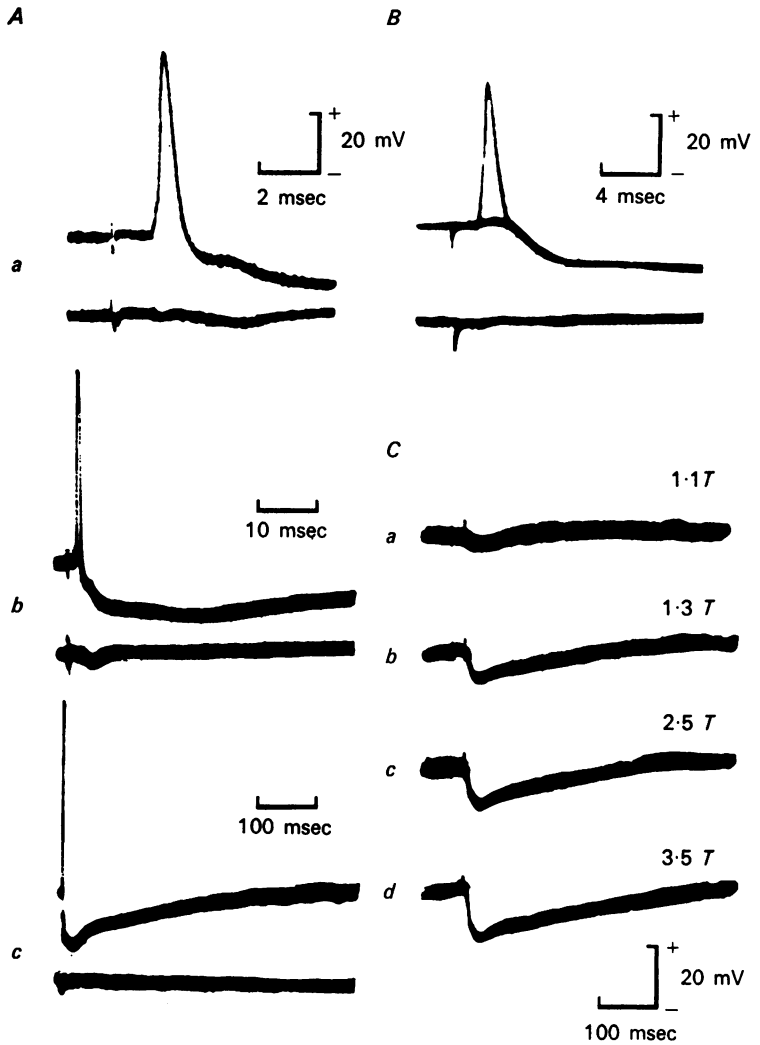

Fig. 2. Intracellular potentials of mitral cells evoked by single l.o.t. stimulation. In *A*, an antidromic spike and a subsequent i.p.s.p. are shown at three different sweep speeds (upper traces). *B* shows mitral cell responses to just threshold stimulation for the antidromic activation of this cell. *C*, Intracellular responses of a mitral cell to l.o.t. stimulation at indicated stimulus intensities (multiples of the threshold stimulus strength (*T*) for eliciting the hyperpolarization). The latency of the antidromic spike of this cell was 2 msec, which later disappeared due to the deterioration. The lower traces in *A* and *B* show the extracellular field potentials recorded just outside of the impaled cell. Spikes retouched.

potential (*cf.* Fig. 4). I.p.s.p.s in mitral cells had slow rise times, attaining their flat peak 10–32 msec (mean 19 ± 4 msec S.D., n = 159) after l.o.t. stimulation, and having a much slower decay time. The total duration of i.p.s.p.s varied among the recordings from different mitral cells within the range of 60–650 msec (mean 300 ± 140 msec S.D., n = 64).

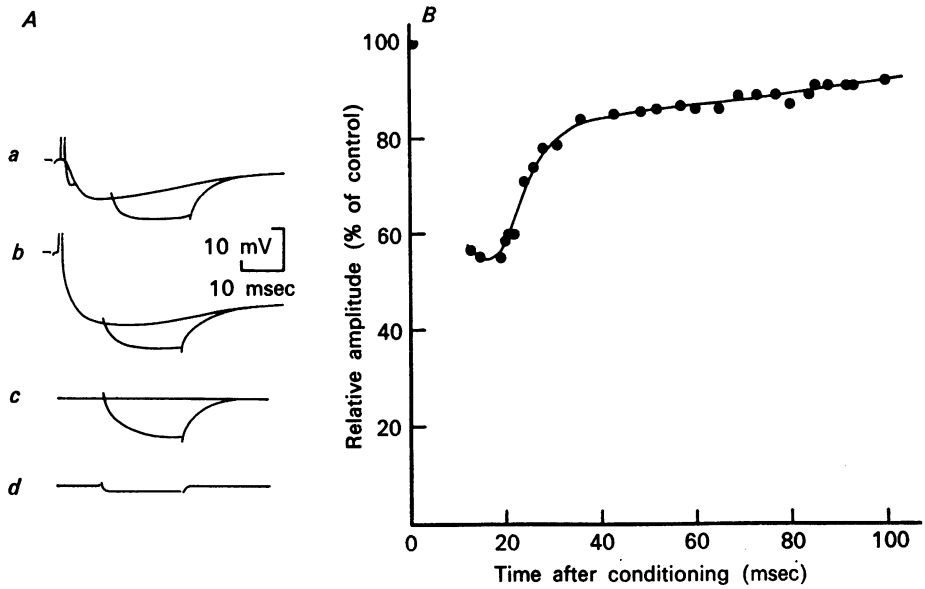

Fig. 3. Membrane conductance changes of a mitral cell during i.p.s.p.s evoked by l.o.t. stimulation. *A*, mitral cell responses to just threshold (*a*) and supramaximal (*b*) stimulation for antidromic activation of this cell. In addition, hyperpolarizing current pulses (19 msec duration, 7×10^{-10} A) were applied through the recording electrode during the i.p.s.p.s and voltage drops produced by the pulses were measured. Fig. *A*, *c* and *d* are the control intracellular (*c*) and extracellular (*d*) voltage drops produced by the same current pulse without conditioning l.o.t. stimulation. *B*, plot of relative amplitudes (as % of the control) of voltage drops produced by test constant current pulses at various times after conditioning l.o.t. stimulation.

Conductance changes of the mitral cell membrane during i.p.s.p.s

The conductance changes of the mitral cell membrane during the i.p.s.p.s were measured by a similar method to that used by Pollen & Lux (1966) for studying the conductance increase in cortical neurones and by Tsukahara & Fuller (1969) in red nucleus neurones. Mitral cell i.p.s.p.s evoked by l.o.t. stimulation have slow time courses similar to those of cortical neurones and red nucleus neurones. A slow time course is advantageous for measuring the conductance changes by applying current pulses of relatively long durations. Fig. 3*A*(*a*) is a superimposed tracing of the i.p.s.p. in a mitral cell elicited by just threshold stimulation for antidromic activation together with the voltage drop produced by a hyperpolarizing current pulse during the falling phase of the i.p.s.p. When this voltage drop was compared with that produced by the same current without the conditioning l.o.t. stimulation (Fig. 3*A*(*c*)) the former had a smaller amplitude and a faster time course than the latter, indicating an increase in membrane conductance of the mitral cell during the i.p.s.p. Using this

method, conductance increases during i.p.s.p.s were observed in all twenty-four mitral cells examined. It can also be seen in Fig. 3*A*(*a*) that the occurrence of the antidromic spike, or afterhyperpolarization of the antidromic spike, had little effect on the testing voltage drop in this period.

When the stimulus strength was increased to supramaximal, as shown in Fig. 3*A*(*b*), the membrane conductance was seen to increase as well as the amplitude of the i.p.s.p. The decrease in membrane resistance of mitral cells measured near the peak of i.p.s.p.s ranged from 20 to 45%. The amplitudes of voltage drops produced by current pulses at different phases of the i.p.s.p.s were measured in twelve mitral cells and plotted against the time after conditioning supramaximal l.o.t. stimulation (Fig. 3*B*). The conductance of the mitral cell membrane increased maximally at or near the peak of the i.p.s.p.s and decreased rapidly thereafter. But, it could still be detected in the declining phase of the i.p.s.p.s (up to 100 msec in Fig. 3*B*).

Effects of hyperpolarizing current injection on mitral cell i.p.s.p.s

The i.p.s.p.s of mitral cells evoked by l.o.t. stimulation were reduced in amplitude and then reversed in polarity by intracellular application of hyperpolarizing current. Fig. 4*A*(*a*) shows the control response of a mitral cell to supramaximal l.o.t. stimulation which consists of an antidromic spike and a long lasting hyperpolarizing i.p.s.p. When a hyperpolarizing current of 0·6 nA was applied internally (*b*), the antidromic spike was blocked and the i.p.s.p. was reduced in amplitude. Increase of the hyperpolarizing current reversed the initial part of the i.p.s.p. into a depolarization, while the later part persisted as a hyperpolarization (*c* and *d*), so that a complex positive-negative wave could be seen. Fig. 4*A*(*e*) and lower trace of *B* shows depolarizing i.p.s.p.s produced by the application of stronger hyperpolarizing current. Here, it can be seen that the depolarizing i.p.s.p.s have quite different time courses from the original hyperpolarizing potential, the former always having shorter time course than the latter. For example, the average latency of the peak of the reversed i.p.s.p. was 7 msec while that of the original hyperpolarizing i.p.s.p. was 19 msec. This asymmetrical reversal suggests that the i.p.s.p. may be produced by an aggregation of inhibitory synapses with different sensitivities to the hyperpolarizing current, which was presumably applied in the soma. In order to compare the vulnerability of the early part of the i.p.s.p. to hyperpolarizing current with that of the later part, the amplitude of the i.p.s.p. at a latency of 7 msec (near the peak of the reversed i.p.s.p., filled circles) and at the latency of 20 msec (near the peak of original i.p.s.p., open circles) are plotted against the injected current in Fig. 4*C*. It can be seen that the early part of the i.p.s.p. (filled circles) is affected more strikingly than the later part by the hyperpolarizing current. For example, the current at the reversal point of the i.p.s.p. at a 7 msec latency was approximately 1·3 nA whereas at a latency of 20 msec it was approximately 1·9 nA. These observations may be explained by the hypothesis that the inhibitory synapses responsible for the mitral cell i.p.s.p.s are not localized on the soma but are distributed widely on the dendrites, and that the early part of the i.p.s.p. is mainly produced by the inhibitory synapses located at or in proximity to the soma, while those located on distal dendrites are responsible for the later part of the i.p.s.p.

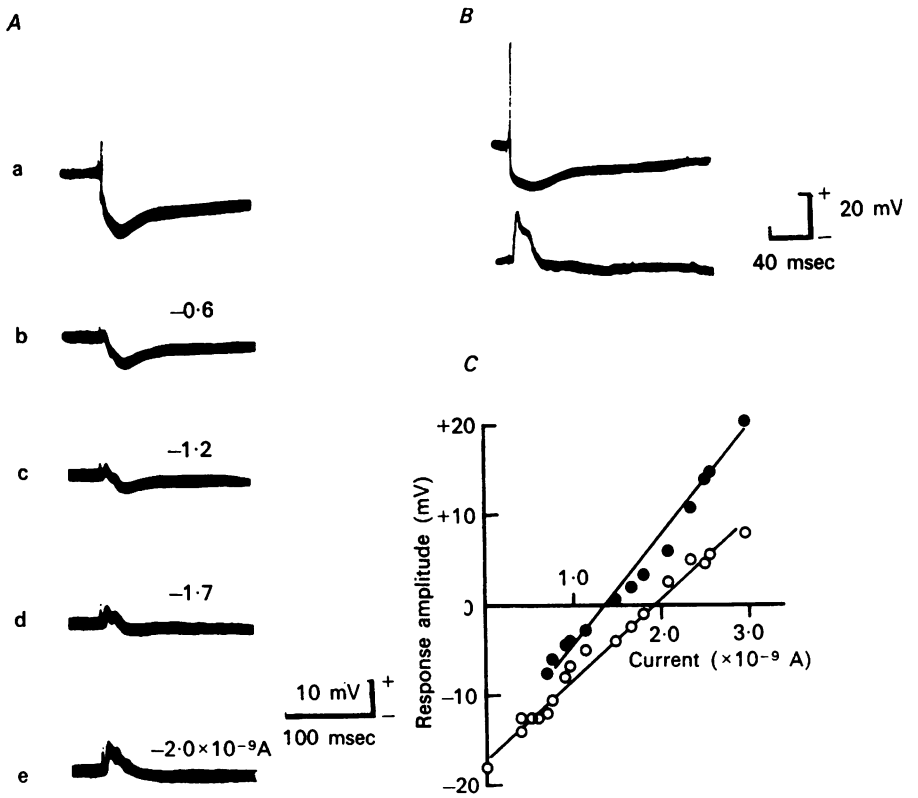

Fig. 4. Intracellular records of i.p.s.p.s from mitral cells during intracellular application of the hyperpolarizing current. Records *A* show the i.p.s.p.s evoked by l.o.t. stimulation during application of progressively increasing hyperpolarizing currents. The control mitral cell response composed of an antidromic spike and a subsequent i.p.s.p. is shown in *a* (the antidromic spike was truncated). In *b–e*, the decrease in amplitude of the i.p.s.p. and then reversal of that occurred when the hyperpolarizing currents were applied through the impaling micro-electrode. The number on the right shoulder of each trace indicates the amount of the applied current. Records in *B* show an i.p.s.p. in another mitral cell elicited by l.o.t. stimulation (upper trace) and a reversed i.p.s.p. during application of a hyperpolarizing current ($2{\cdot}9 \times 10^{-9}$ A). *C* shows a plot of the amplitude of the i.p.s.p. at a latency of 6 msec (filled circles) and 20 msec (open circles) following l.o.t. stimulation (ordinate) against the intracellularly applied currents (abscissa), from the same cell as *B*. Spikes retouched.

Latency of mitral cell responses

Fig. 5*A* shows the antidromic spike potential in a mitral cell elicited by l.o.t. stimulation (middle trace) together with the simultaneously recorded field potential in the g.c.l. (upper trace). The field potential in the g.c.l. consists of an early negative wave with onset latency from 0·9 to 1·6 msec and duration of about 1 msec (component 1) which was followed by a large positive wave (component 2) (*cf.* Fig. 1*B*). The interval between the onset time of component 1 (dotted line in Fig. 5*A*) and that of the antidromic spike (upward arrow) was measured in 136 mitral cells and the frequency distribution of the time interval was plotted in Fig. 5*C*. As shown by the blank columns, the range was 0·0–1·0 msec (mean $0{\cdot}5 \pm 0{\cdot}2$ msec S.D., $n = 136$)

indicating that component 1 corresponds well in time with the occurrence of antidromic activation of mitral cells. This observation confirms the postulate that component 1 can be attributed to the antidromic spike potentials of mitral cell somata (Rall & Shepherd, 1968). In Fig. 5*B*, the superimposed tracings of the normal i.p.s.p. and the reversed i.p.s.p. produced by application of the hyperpolarizing current (middle traces) are shown together with the field potential in the g.c.l. (upper trace). The onset time of the i.p.s.p. was determined as the divergence

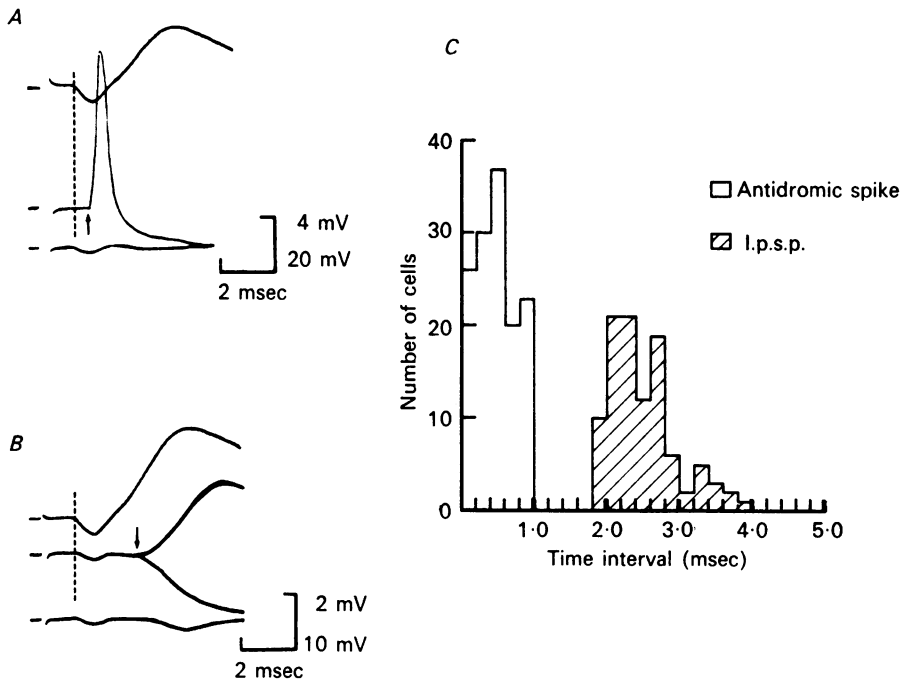

Fig. 5. Responses of mitral cells to l.o.t. stimulation. *A*, intracellularly recorded potential change in a mitral cell (middle trace) together with the field potential in the g.c.l. (upper trace); the onset of the antidromic spike is marked by an upward arrow. *B*, superimposed tracings of the original hyperpolarizing i.p.s.p. in another mitral cell and the reversed i.p.s.p. during passage of the hyperpolarizing current of 2×10^{-9} A (middle traces). A downward arrow marks the diverging point of the above two traces. The dotted lines mark the onset of the component 1 of the field potential in g.c.l. in *A* and *B*. The lower traces in *A* and *B* show the extracellular field potential recorded just outside of the impaled cell. *C*, frequency distribution of the time interval between onset of component 1 and that of the antidromic spike (blank columns) or the i.p.s.p. (cross hatched columns). In this Figure and Figs. 6, 7, 8 and 9, upper voltage calibrations are for the field potentials in the g.c.l.; lower ones are for intracellular potentials and extracellular potentials just outside the impaled cells.

point of these two tracings (marked by a downward arrow). When hyperpolarizing current injection was not successful in reversing the polarity of the i.p.s.p.s, the onset time was determined as the divergence point of the superimposed tracings of the hyperpolarizing i.p.s.p. and the extracellular field potential recorded just outside the cell. The onset latency thus measured ranged from 3·0 to 5·5 msec (mean $3{\cdot}9 \pm 0{\cdot}6$ msec S.D., $n = 105$). This latency contains, however, the conduction

time of the antidromic spike from the stimulating site along the mitral cell axon to the soma of the mitral cell. This conduction time varied considerably from experiment to experiment, because of difference in the distance from the stimulating to the recording site, and also because of the relatively slow conduction velocity of mitral cell axons (about 10 m/sec; Mori, K. & Takagi, S. F., unpublished observation). In order to exclude this conduction time, the interval between the onset time of the component 1, which signals the arrival of the antidromic spike in the somata of the mitral cells with fastest axonal conduction velocities, and the onset time of the i.p.s.p. elicited by supramaximal l.o.t. stimulation was measured. This time interval ranged from 1·8 to 3·9 msec in 102 mitral cells (mean $2{\cdot}4 \pm 0{\cdot}4$ msec S.D.) (cross-hatched columns in Fig. 5*C*), indicating that mitral cell i.p.s.p.s are not produced monosynaptically, but there may be inhibitory interneurones responsible for the i.p.s.p.s. As will be shown later in Fig. 7, the onset latency of the mitral cell i.p.s.p. was progressively decreased as the intensity of l.o.t. stimulation was increased. This also suggests that interneurones may be interpolated in the inhibitory pathway.

Responses of g.c.l. neurones to l.o.t. stimulation

When a micro-electrode was in the g.c.l., it often penetrated cells whose responses to the l.o.t. stimulation were entirely different from those of mitral cells. Many of the recordings from these g.c.l. cells showed an element of damage due to micropipette penetration as evidenced by a rapid decrease in the resting membrane potential. The following observations were derived from comparatively stable recordings from sixty-three g.c.l. cells with resting membrane potentials from -40 to -64 mV. An example of the responses of a g.c.l. cell is shown in Fig. 6*A*(*b*) together with the simultaneously recorded field potential in the middle area of the g.c.l. (*a*). Supramaximal l.o.t. stimulation elicited a slow depolarization with superimposed spikes. This depolarization corresponded in time to component 2 of the g.c.l. field potential although the depolarization lasted longer than component 2. Such a depolarizing responses was a common feature of g.c.l. cells and no antidromic spikes were recorded from them following l.o.t. stimulation.

Fig. 6*B* are the records from another g.c.l. cell showing a depolarization of about 14 mV amplitude and 60 msec duration with three superimposed spikes (*b*). When a hyperpolarizing current of 0·5 nA was applied intracellularly (*c*), the amplitude of the depolarization increased to about 20 mV, indicating that this depolarization is not produced by a disinhibitory mechanism but rather by an activation of the excitatory synapses on the g.c.l. cell. It can also be seen in (*c*) that the full spikes were blocked and only the partial spikes remained. Increase of the hyperpolarizing current to 0·9 nA (*d*) augmented the amplitude of the e.p.s.p. and blocked the partial spikes except the first one.

In Fig. 6*C*, the e.p.s.p. was recorded together with the field potential in the middle area of g.c.l. with faster sweep speed in order to measure the time interval between the onset of component 1 (downward arrow) and the onset of the e.p.s.p. (upward arrow). As shown in *D*, the time interval ranged from 1·2 to 2·3 msec (mean $1{\cdot}8 \pm 0{\cdot}3$ msec S.D., $n = 23$) indicating that the onset of the e.p.s.p. in g.c.l. cells is about 0·6 msec earlier than that of the i.p.s.p. in mitral cells (*cf.* Fig. 5).

Dendrodendritic synaptic pathways

It has been suggested that granule cells are the inhibitory interneurones mediating mitral cell inhibition (e.g. Shepherd, 1963) deep in the olfactory bulb. Furthermore, Rall & Shepherd (1968) postulated that antidromic activation of mitral cell dendrites cause synaptic excitation of the peripheral processes of granule cells through mitral-to-granule dendrodendritic excitatory synapses, and that component 2 of the field potential is produced by the flow of extracellular current from the deep dendrites of the granule cells radially outward to the synaptically depolarized peripheral

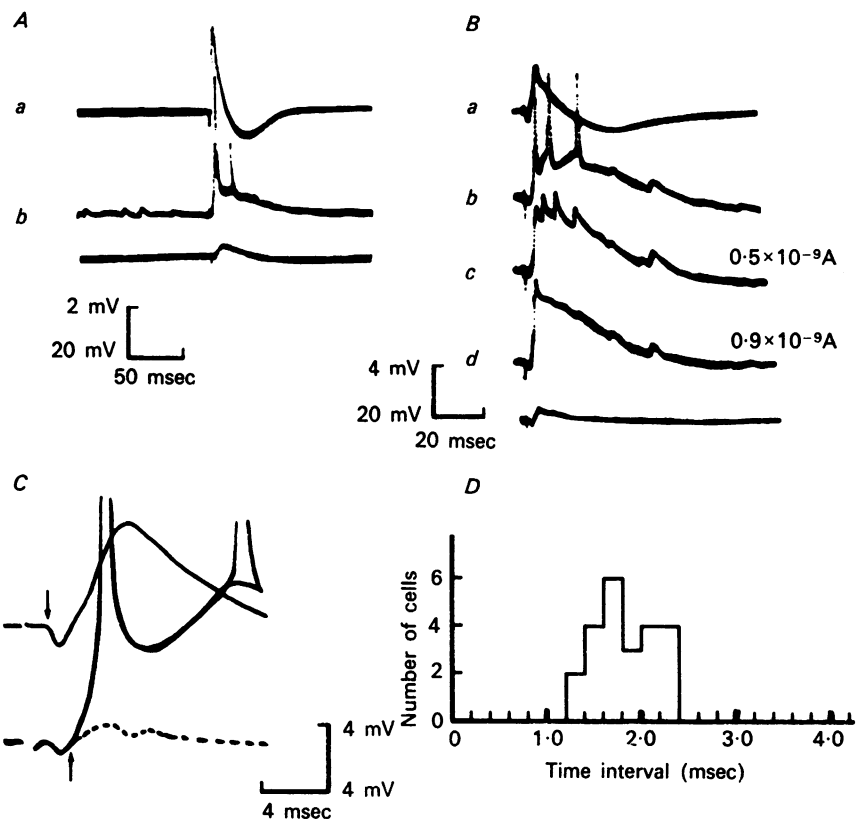

Fig. 6. E.p.s.p.s in g.c.l. cells produced by the l.o.t. stimulation. *A*, intracellular potentials in a g.c.l. cell (*b*) together with the simultaneously recorded field potential in the middle area of the g.c.l. (*a*). *B*, changes in the intracellular potentials in another g.c.l. cell produced by the application of hyperpolarizing current; e.p.s.p.s and superimposed spikes were induced by supramaximal l.o.t. stimulation in *b*. The simultaneously recorded field potential in the middle area of the g.c.l. is shown in *a*. Hyperpolarizing currents of $0{\cdot}5 \times 10^{-9}$ A and $0{\cdot}9 \times 10^{-9}$ A were applied in *c* and *d* respectively. The lowest traces in *A* and *B* are the field potentials taken just after withdrawal. *C*, superimposed tracings of the intracellular and just extracellular (interrupted line) recordings from the other g.c.l. cell (lower traces) together with the field potential in the middle area of the g.c.l. (upper trace). Upward arrow indicates the diverging point of the above two traces. Hyperpolarizing current of $0{\cdot}2 \times 10^{-9}$ A was applied in order to increase the amplitude of the e.p.s.p. *D*, frequency distribution of the time interval between onset of the component 1 and that of the e.p.s.p. in g.c.l. cell. Spikes retouched.

processes of the granule cells in the e.p.l. If the above hypotheses are correct, the e.p.s.p. of the granule cell and component 2 of the field potential in the g.c.l. should show a parallel behaviour under all conditions and both of them should have a positive correlation with the i.p.s.p. in the mitral cell.

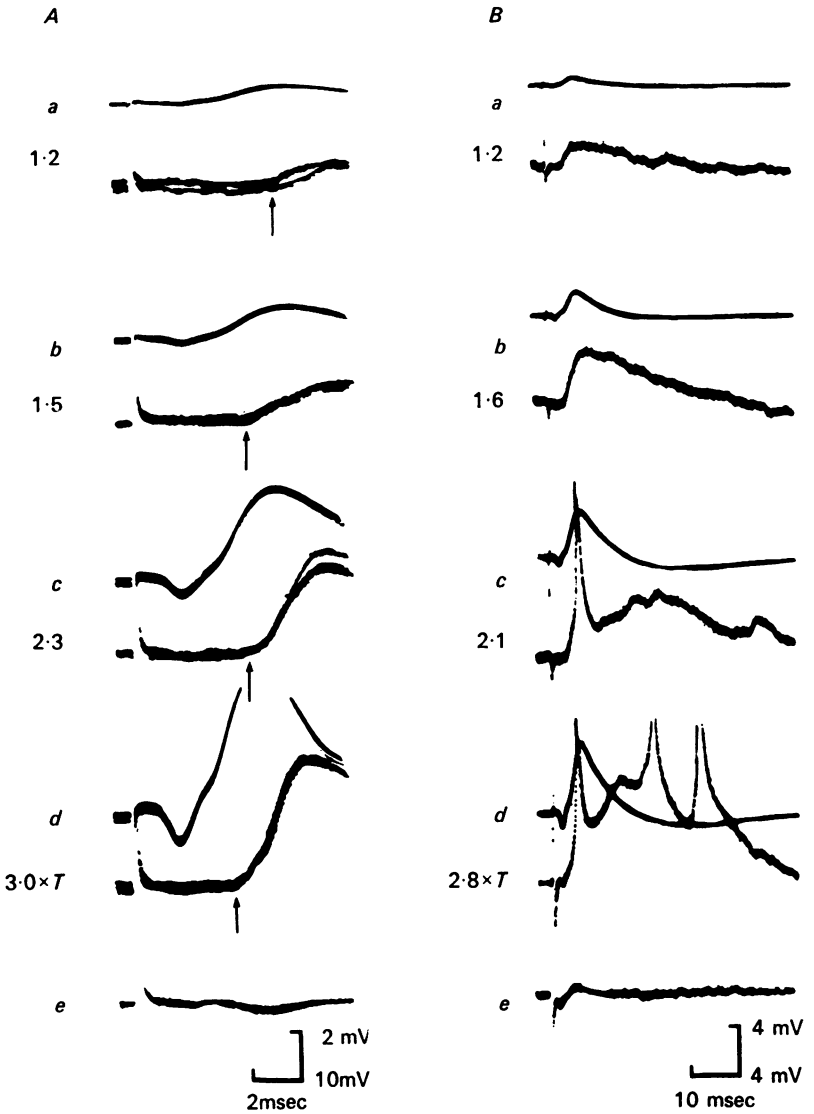

Fig. 7. Comparison of intracellular potentials and field potentials elicited by l.o.t. stimulations at various intensities. *A*, *a–d*, Reversed i.p.s.p.s in a mitral cell (lower traces) together with the simultaneously recorded field potentials in the middle area of g.c.l. (upper traces); hyperpolarizing current of about 5×10^{-9} A was applied internally throughout these recordings. The onsets of the reversed i.p.s.p.s were indicated by the upward arrows; *e*, extracellular field potential recorded just after withdrawal. *B*, *a–d*, e.p.s.p.s in a g.c.l. cell (lower traces) together with the field potential in the middle area of g.c.l. (upper traces); *e*, extracellular field potential recorded just outside of the cell. The stimulus intensities were indicated by the multiples of the threshold strength (*T*) for eliciting the component 2 the field potential in the g.c.l. Spikes retouched.

In order to test the above hypotheses two experiments were performed. First the intensities of l.o.t. stimulation were graded while the field potential in the g.c.l. and the synaptic potentials in the mitral or g.c.l. cells were recorded simultaneously. Graded stimulation of the l.o.t. caused graded i.p.s.p.s in the mitral cells and graded field potentials in the g.c.l. An example of simultaneous recordings of them is shown in Fig. 7*A*, where the i.p.s.p.s were reversed in polarity by the hyperpolarizing current. The threshold strength for eliciting the field potential in the g.c.l. was almost equal to that for eliciting the mitral cell i.p.s.p. (*a*). As l.o.t. stimulation was strengthened the amplitude of the i.p.s.p. increased in parallel with that of the field potential (both component 1 and 2). It can also be seen in Fig. 7*A* that the latency of the i.p.s.p. progressively decreased (from 5·8 msec to 4·2 msec in this Figure) as the

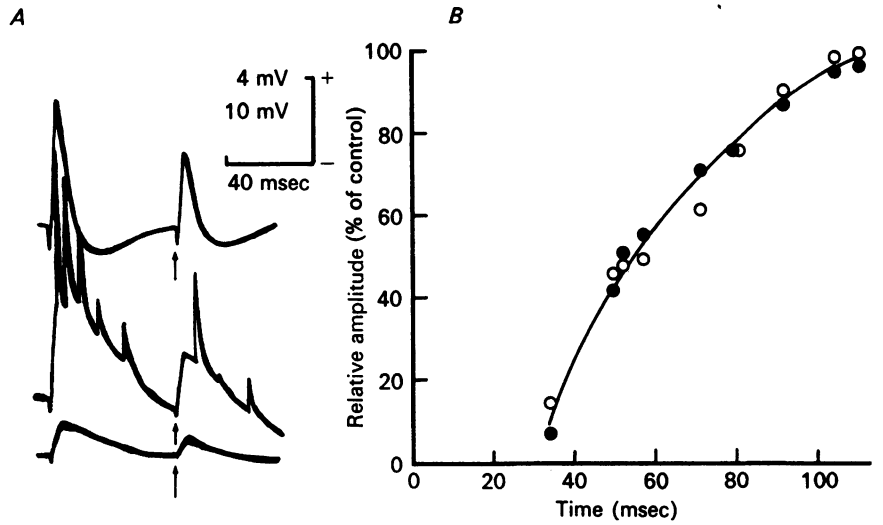

Fig. 8. Intracellular records from a g.c.l. cell. *A*, e.p.s.p.s and superimposed partial spikes evoked by the paired l.o.t. shocks of equal intensity (middle trace) together with the simultaneously recorded field potential in the middle area of the g.c.l. (upper trace). The lowest trace shows the extracellular field potential recorded just after withdrawal. The time of test stimuli was indicated by the upward arrows. *B*, plot of relative amplitudes of the e.p.s.p.s in the same cell as in *A* (open circles) and field potentials in the g.c.l. (filled circles) elicited by the test l.o.t. volley (ordinate) against the interval between the conditioning and test stimulus (abscissa).

stimulus intensity was increased. Not only is the amplitude of the i.p.s.p. in the mitral cell correlated with that of the field potential in the g.c.l., but also the amplitude of the e.p.s.p. in the g.c.l. cell. This is demonstrated in Fig. 7*B*, where the upper traces show the field potentials in the g.c.l. and the lower traces are intracellular records from a g.c.l. cell. As the intensity of l.o.t. stimulation was gradually increased from subthreshold strength, the field potential and the e.p.s.p. appeared simultaneously at an almost equal threshold intensity (*a*) and then varied together in amplitude (*b–d*).

The second experiment to test the hypothesis of the dendrodendritic pathways for activation of the granule cells and subsequent inhibition of mitral cells was to use paired l.o.t. volleys of equal intensity. A conditioning l.o.t. stimulation elicits

large i.p.s.p.s in the mitral cells, which would prevent test antidromic spikes from invading the mitral cell somata or dendrites. If the granule cells are activated through the mitral-to-granule dendrodendritic pathway, conditioning l.o.t. volleys would thus depress the test e.p.s.p.s in the granule cells. On the other hand, if the granule cells are activated through an axon collateral pathway, the test e.p.s.p.s in granule cells should not be depressed by the conditioning l.o.t. stimulation because the axon collateral pathway would not be blocked by the i.p.s.p.s in the mitral cell somata or dendrites. In most of the g.c.l. cells (twenty-three out of twenty-six cells), the e.p.s.p. responses to test l.o.t. stimulation were markedly depressed by the conditioning l.o.t. stimulation, as shown in Fig. 8*A* (middle trace). In Fig. 8*B*, the

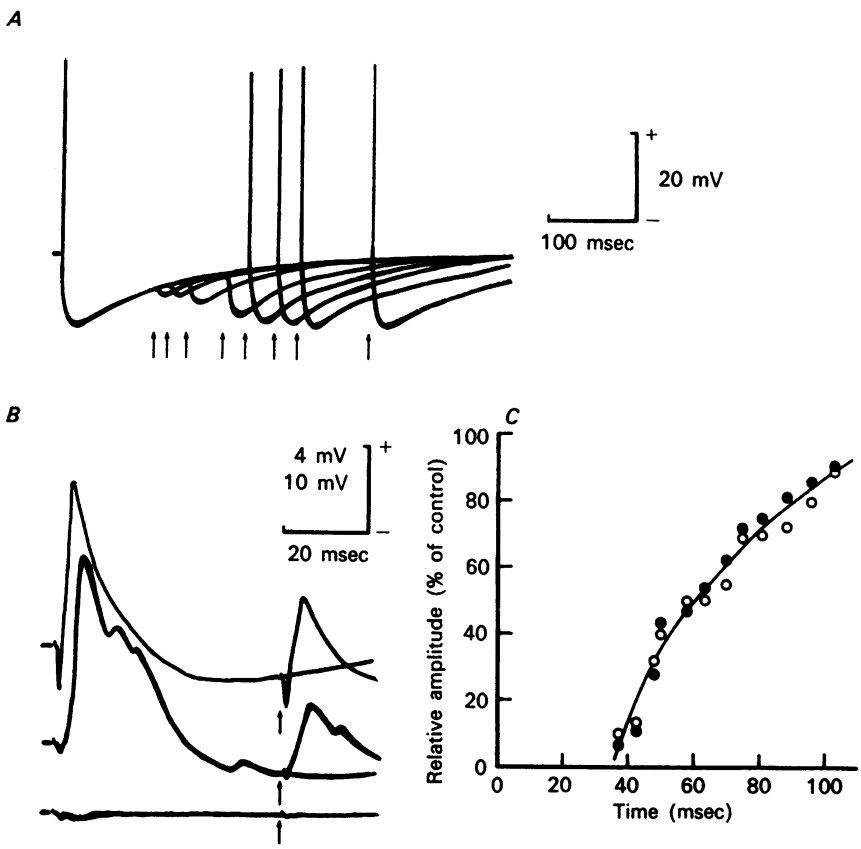

Fig. 9. Intracellular records from mitral cells. *A*, paired shocks of equal intensity were delivered to the l.o.t. at various intervals between conditioning and test stimulus; eight traces of responses were superimposed. *B*, reversed i.p.s.p.s elicited by the paired l.o.t. shocks of equal intensity during hyperpolarizing current application (4×10^{-9} A) (middle traces) together with simultaneously recorded field potential in the g.c.l. (upper traces); KCl electrode; two traces with and without test stimuli were superimposed; the lowest trace indicates the extracellular field potential recorded just after withdrawal; the times of test stimulus were indicated by upward arrows in *A* and *B*. *C*, plot of relative amplitudes of the reversed i.p.s.p.s (open circles) and field potentials in the g.c.l. (filled circles) evoked by the test l.o.t. stimulation (ordinate) against the interval between the conditioning and test stimulus (abscissa); from the same cell as in *B*.

relative amplitudes of the test l.o.t.-evoked e.p.s.p.s in the g.c.l. cells (indicated by open circles) and of the test l.o.t.-evoked field potentials (component 2) in the g.c.l. (indicated by filled circles) following conditioning l.o.t. stimulation are plotted against the interval between the conditioning and test stimulus. In this experiment, the intensity of conditioning stimulation was equal to that of the test stimulation. It can be seen in this Figure that the depression curves have quite similar time courses.

Fig. 9*A* shows the mitral cell responses to paired l.o.t. stimulation. When the interval between the conditioning and test stimulation was decreased, the antidromic spikes of the test volleys were blocked and the test i.p.s.p.s were markedly reduced in amplitude which confirms Nicoll's results (1969). In *B*, reversed i.p.s.p.s in a mitral cell are shown together with the field potentials in the g.c.l. elicited by the paired l.o.t. volleys. It can be seen in this Figure that the amplitudes of both the test i.p.s.p.s and the g.c.l. field potentials were depressed in almost equal degree. The relative amplitudes of the test i.p.s.p.s (open circles) and those of the test field potentials (component 2, filled circles) were plotted against the intervals between the conditioning and test stimuli (*C*). As seen in *C*, the depression curves had similar time courses, just as those of the e.p.s.p.s in g.c.l. cells and the g.c.l. field potentials (Fig. 8*B*) under conditions of paired l.o.t. stimuli of equal intensity. It should be noted that this depression of test l.o.t.-evoked i.p.s.p.s were observed in all the forty-six mitral cells which were examined by paired l.o.t. volleys. These observations thus strongly support the hypothesis of the dendrodendritic pathways for activation of granule cells and subsequent inhibition of the mitral cells.

DISCUSSION

Mitral cell i.p.s.p.s

The present results have confirmed the previous observations that l.o.t. stimulation causes large amplitude i.p.s.p.s in the mitral cells (Yamamoto *et al.* 1963; Phillips *et al.* 1963; Nicoll, 1969; Reese & Shepherd, 1972). These i.p.s.p.s had essentially similar characteristics as the i.p.s.p.s of the other neurones, i.e. they were accompanied by a conductance increase of the mitral cell membrane (*cf.* Nicoll, 1969) and were decreased in amplitude and then reversed in polarity by intracellular application of hyperpolarizing current. A characteristic feature of the mitral cell i.p.s.p.s is their long duration, which is comparable to those of hippocampal pyramidal cells (e.g. Kandel, Spencer & Brinley, 1961) cortical pyramidal cells (e.g. Phillips, 1959) and thalamic neurones (e.g. Purpura & Cohen, 1962). It should also be noted that the conductance increase of the mitral cell membrane lasted up to 100 msec during the i.p.s.p.s when supramaximal stimulation was applied to the l.o.t.

An asymmetrical reversal of the i.p.s.p.s by intracellular injection of hyperpolarizing current has been observed in cerebellar Purkinje cells following parallel fibre stimulation (Eccles, Llinás & Sasaki, 1966). This has been ascribed to the distribution of inhibitory synapses both on the somata and dendrites of the Purkinje cell. In the present experiment, the reversed i.p.s.p.s of the mitral cells produced by hyperpolarizing current application were found quite asymmetrical in shape compared to the original i.p.s.p.s, the time course of the former being always much shorter than that of the latter. If as seems likely (see below) the inhibitory synapses

on the mitral cell are not localized on the soma but distributed widely both on the soma and dendrites, then the asymmetrical reversal of the i.p.s.p.s may be interpreted as follows. The hyperpolarizing current application may produce a non-uniform change in the membrane potential of the mitral cell. Thus, the i.p.s.p.s produced by the synapses located near the soma may be easily reversed in polarity by the hyperpolarizing current application because the soma is the presumed site of current injection. On the other hand, the i.p.s.p.s produced by the synapses on distal dendrites may not be reversed due to insufficient potential change of the subsynaptic membrane. Comparison of the sensitivity to an applied current between the early part and the later part of the mitral cell i.p.s.p.s suggested that the former may be mainly produced by the inhibitory synapses located on the soma or proximal dendrites, and the latter by those located on the remote dendrites. It is known from a number of electron microscopic investigations that granule cell synapses are not localized solely on the somata of the mitral cells but distributed widely on both soma and dendrites (e.g. Price & Powell, 1970*b*). Most of the synapses are in fact located on mitral cell dendrites with relatively small diameters. Furthermore, nearly all the synapses found on mitral cell dendrites and somata are reciprocal synapses with the gemmules of granule cell peripheral processes, except in or around the glomerular region (Price & Powell, 1970*b*; Reese & Shepherd, 1972). Using immunocytochemical methods, it has also been reported that glutamate decarboxylase (GAD), the enzyme that synthesizes the neurotransmitter γ-aminobutyric acid, is located in the gemmules of granule cells and that, in some instances, the GAD-positive gemmules appeared to line up adjacent to the secondary dendrites of the mitral cell (Ribak, Vaughn, Saito, Barber & Roberts, 1977).

Responses of granule layer cells to l.o.t. stimulation

According to histological studies, there exist two types of neuronal somata in the g.c.l.: many granule cells (*cf.* Fig. 1*B*) and a smaller number of short-axon cells. Thus, micro-electrodes may encounter short-axon cells as well as granule cells. If the short-axon cells receive excitatory inputs from axon collaterals of the mitral cells or centrifugal fibres running in the l.o.t., they may also show e.p.s.p.s following l.o.t. stimulation. Because of the axon collateral pathway (or centrifugal fibre pathway), the test l.o.t. evoked e.p.s.p.s in these short-axon cells should not be depressed by the conditioning l.o.t. stimulation. In fact, a few g.c.l. cells were encountered which did not show depression of test l.o.t.-evoked responses following conditioning l.o.t. stimulation (type 2 g.c.l. cell, unpublished observations). In the present study, such cells were excluded and all the g.c.l. cells presented in this communication showed clear depression of test l.o.t.-evoked e.p.s.p.s by the conditioning l.o.t. stimulation (type 1 g.c.l. cell). Furthermore, these type 1 g.c.l. cells showed the following responses which would be expected in granule cells:

(1) The e.p.s.p.s elicited by l.o.t. stimulation corresponded in time with component 2 of the field potential in the g.c.l. which appears to be produced by depolarization of granule cells.

(2) When the intensity of l.o.t. stimulation was increased from a subthreshold strength for activating mitral cell axons, the amplitude of the e.p.s.p.s progressively increased in parallel with that of the g.c.l. field potential (both components 1 aud 2).

(3) The test l.o.t.-evoked e.p.s.p.s were depressed not only by conditioning l.o.t. stimulation but also by conditioning stimulation of the anterior commissure (Mori & Takagi, 1978) or deep lying structure of the prepiriform cortex (Mori, K. & Takagi, S. F., unpublished observations), both of which cause i.p.s.p.s in the mitral cells.

(4) Moreover, the depression of the test l.o.t.-evoked e.p.s.p.s following conditioning stimulation of one of these sites is similar in time course to the depression of the component 2 of the field potential elicited by the test l.o.t. stimulation (*cf.* Mori & Takagi, 1978).

These observations strongly suggest that the g.c.l. cells described in this study are the granule cells which receive excitatory synaptic input from the mitral cell dendrites. Because of the lack of the morphological identification of the impaled g.c.l. cells, we, nevertheless, cannot rule out the possibility that impaled g.c.l. cells are a type of deep short-axon cell, if the dendrites of such cells should have reciprocal synaptic interactions with the mitral cells. However, there are as yet no anatomical reports of such reciprocal synapses between the dendrites of mitral cells and short-axon cells.

The time interval between the onset of the field potential produced by the antidromic spike potentials of mitral cell somata and the onset of the e.p.s.p.s in presumed granule cells ranged from 1·2 to 2·3 msec (mean 1·8 msec). This time interval may contain not only the synaptic delay from a mitral cell dendrite to the presumed granule cell but also the time required for active or passive depolarization of the mitral cell dendrites by the invasion of antidromic spike potentials. In the theoretical model of the mitral cell presented by Rall & Shepherd (1968), depolarization of the mitral cell dendrites occurs late in period I and during period II (see Fig. 7 of Rall & Shepherd, 1968), i.e. depolarization of the mitral cell dendrites sufficient for activation of the dendrodendritic synapse occurs presumably about 1 msec after the onset of component 1 of the field potential in the g.c.l. Thus the time interval between the onset of component 1 of the field potential and that of the e.p.s.p.s in presumed granule cells is compatible with the postulate that the granule cells are monosynaptically excited by the mitral-to-granule dendrodendritic synapses following l.o.t. stimulation; the time interval may include the time for depolarization of mitral cell dendrites (about 1 msec) and that for a synaptic delay from the mitral cell dendrites to the granule cells (about 0·8 msec).

It is well known that the granule cells in the olfactory bulb are axonless neurones analogous to the amacrine cells in the retina (Cajal, 1955). The presumed granule cells in this study showed two types of spike potentials, large amplitude spikes and small partial spikes. Similar types of spike potentials have been reported in mudpuppy amacrine cells, in which large and small spikes are assumed to be somatic and dendritic impulse activity respectively (Miller & Dacheux, 1976). The large amplitude spikes in presumed granule cells are much more easily blocked by intracellular application of a hyperpolarizing current than small spikes (*cf.* Fig. 6*B*), which suggests that the large spikes may be elicited in the somata or proximal dendrites while the small spikes may be produced in the peripheral processes. It has been suggested by Rall & Shepherd (1968) that at the dendrodendritic synapses between the mitral and granule cells, synaptic activation would not require a presynaptic action potential; the depolarization of the presynaptic membrane

itself could activate the dendrodendritic synapse. Such a depolarization produced at individual gemmules would cause sufficient electrotonic depolarization in the neighbouring gemmules of the same granule cell for activating the dendrodendritic inhibitory synapses. However, it can be assumed that there would be a considerable damping of the electrotonically spread depolarization at the gemmules located far from the synaptically depolarized gemmules. The spike activity of the peripheral process of granule cells may function as a booster for depolarizing the remote gemmules of the same cell.

Dendrodendritic synaptic interactions between mitral cell dendrites and peripheral processes of granule cells

The excitatory nature of dendrodendritic synapses from the mitral to the granule cells and the inhibitory nature of those from the granule to the mitral cells are consistent with the morphology of the reciprocal synapses between the two cells (Fig. 1*C*); the mitral-to-granule synapse has an asymmetrical synaptic thickening and spheroidal synaptic vesicles, while the granule-to-mitral synapse has a symmetrical synaptic thickening and flattened vesicles (Rall *et al.* 1966; Price & Powell, 1970*a*).

It is interesting to note that these dendrodendritic synaptic interactions may be well suited for developing rhythmic activity in the olfactory bulb (Rall & Shepherd, 1968; Mori & Takagi, 1977*a*). When the mitral cells are excited, they would activate the granule cells through the mitral-to-granule dendrodendritic excitatory synapses. The activated granule cells would in turn inhibit the mitral cells. Thus the dendrodendritic reciprocal synapse provides a negative feed-back mechanism. When the mitral cells are inhibited by this negative feedback mechanism, excitatory input would fail to activate not only the cell itself but also the mitral-to-granule dendrodendritic synapses. Therefore the activity of granule cells would decrease and the amount of the synaptic inhibition of mitral cells due to the feed-back mechanism would also decrease. When the mitral cells receive excitatory inputs at this time, they would activate the dendrodendritic reciprocal synapses again. In this way, successive excitatory input to the mitral cells would cause rhythmic activity of the mitral and granule cells. For example, repetitive l.o.t. stimulation causes alternation in the amplitude of successive i.p.s.p.s in mitral cells and e.p.s.p.s in presumed granule cells (Mori, Kogure, & Takagi, 1977). The characteristic rhythmic activity in the olfactory bulb during odor stimulation (Adrian, 1950) may also be based on the dendrodendritic interactions between mitral and granule cells (Rall & Shepherd, 1968; Mori & Takagi, 1977*a*).

We are indebted to Dr G. M. Shepherd and Dr T. V. Getchell for their helpful advice on the preparation of this manuscript and to Mrs Yokozawa, Miss Yajima and Mrs Wada for their technical assistance.

REFERENCES

ADRIAN, E. D. (1950). The electrical activity of the mammalian olfactory bulb. *Electroenceph. clin. Neurophysiol.* **2**, 377–388.

ANDRES, K. H. (1965). Der Feinbau des Bulbus Olfactorius der Ratte unter besonderer Berücksichtigung der synaptishen Verbindungen. *Z. Zellforsh. mikrosk. Anat.* **65**, 530–561.

CAJAL, S. R. (1955). *Studies on the Cerebral Cortex.* (Translated by Kraft, L. M.) London: Lloyd-Luke.

COOMBS, J. S., ECCLES, J. C. & FATT, P. (1955). The specific ionic conductance and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. *J. Physiol.* **130**, 326–373.

ECCLES, J. C. (1964). *The Physiology of Synapses.* Berlin: Springer.

ECCLES, J. C., LLINÁS, R. & SASAKI, K. (1966). Intracellularly recorded responses of the cerebellar Purkinje cells. *Expl Brain Res.* **1**, 161–183.

GREEN, J. D., MANCIA, M. & BAUMGARTEN, R. VON. (1962). Recurrent inhibition in the olfactory bulb. I. Effects of antidromic stimulation of the lateral olfactory tract. *J. Neurophysiol.* **25**, 467–488.

HIRATA, Y. (1964). Some observations on the fine structure of the synapses in the olfactory bulb of the mouse, with particular reference to the atypical synaptic configuration. *Arch. histol. jap.* **24**, 293–302.

KANDEL, E. R., SPENCER, W. A. & BRINLEY, F. J. (1961). Electrophysiology of hippocampal neurons. I. Sequential invasion and synaptic organization. *J. Neurophysiol.* **24**, 225–242.

MILLER, R. F. & DACHEUX, R. (1976). Dendritic and somatic spikes in mudpuppy amacrine cells: identification and TTX sensitivity. *Brain Res.* **104**, 157–162.

MORI, K., KOGURE, S. & TAKAGI, S. F. (1977). Alternating responses of olfactory bulb neurons to repetitive lateral olfactory tract stimulation. *Brain Res.* **133**, 150–155.

MORI, K. & TAKAGI, S. F. (1975). Spike generation in the mitral cell dendrite of the rabbit olfactory bulb. *Brain Res.* **100**, 685–689.

MORI, K. & TAKAGI, S. F. (1977*a*). Inhibition in the olfactory bulb; dendrodendritic interactions and their relation to the induced waves. *Food Intake and Chemical Senses*, pp. 33–43. Tokyo: Tokyo Univ. Press.

MORI, K. & TAKAGI, S. F. (1977*b*). Inhibitory potentials in mitral cells of the rabbit olfactory bulb. *Proc. int. Union Physiol. Sci.* **13**, 527.

MORI, K. & TAKAGI, S. F. (1978). Activation and inhibition of olfactory bulb neurones by anterior commissure volleys in the rabbit. *J. Physiol.* **279**, 589–604.

NICOLL, R. A. (1969). Inhibitory mechanisms in the rabbit olfactory bulb: dendrodendritic mechanisms. *Brain Res.* **14**, 157–172.

OCHI, J. (1963). Olfactory bulb responses to antidromic olfactory tract stimulation in the rabbit. *Jap. J. Physiol.* **13**, 113–128.

PHILLIPS, C. G. (1959). Actions of antidromic pyramidal volleys on single Betz cells in the cat. *Q. Jl exp. Physiol.* **44**, 1–25.

PHILLIPS, C. G., POWELL, T. P. S. & SHEPHERD, G. M. (1963). Responses of mitral cells to stimulation of the lateral olfactory tract in the rabbit. *J. Physiol.* **168**, 65–88.

POLLEN, D. A. & LUX, H. D. (1966). Conductance changes during inhibitory postsynaptic potential in normal and strychninized cortical neurons. *J. Neurophysiol.* **29**, 369–381.

PRICE, J. L. & POWELL, T. P. S. (1970*a*). The synaptology of the granule cells of the olfactory bulb. *J. cell Sci.* **7**, 125–155.

PRICE, J. L. & POWELL, T. P. S. (1970*b*). The mitral and short axon cells of the olfactory bulb. *J. cell Sci.* **7**, 631–651.

PURPURA, D. P. & COHEN, B. (1962). Intracellular recording from thalamic neurons during recruiting responses. *J. Neurophysiol.* **25**, 621–635.

RALL, W. & SHEPHERD, G. M. (1968). Theoretical reconstruction of field potentials and dendrodendritic synaptic interactions in olfactory bulb. *J. Neurophysiol.* **31**, 884–915.

RALL, W., SHEPHERD, G. M., REESE, T. S. & BRIGHTMAN, M. W. (1966). Dendrodendritic synaptic pathway for inhibition in the olfactory bulb. *Expl Neurol.* **14**, 44–56.

REESE, T. S. & SHEPHERD, G. M. (1972). Dendrodendritic synapses in the central nervous system. *Structure and Function of Synapses*, ed. PAPPAS, G. D. & PURPURA, D. P. pp. 121–136, New York: Raven.

RIBAK, C. E., VAUGHN, J. E., SAITO, K., BARBER, R. & ROBERTS, E. (1977). Glutamate decarboxylase localization in neurons of the olfactory bulb. *Brain Res.* **126**, 1–18.

SHEPHERD, G. M. (1963). Neuronal systems controlling mitral cell excitability. *J. Physiol.* **168**, 101–117.

SHEPHERD, G. M. (1972). Synaptic organization of the mammalian olfactory bulb. *Physiol. Rev.* **52**, 864–917.

TSUKAHARA, N. & FULLER, D. R. G. (1969). Conductance changes during pyramidally induced postsynaptic potentials in the red nucleus neurons. *J. Neurophysiol.* **32**, 35–42.

WILLEY, T. J. (1973). The ultrastructure of the cat olfactory bulb. *J. comp. Neurol.* **152**, 211–232.

YAMAMOTO, C., YAMAMOTO, T. & IWAMA, K. (1963). The inhibitory system in the olfactory bulb studied by intracellular recording. *J. Neurophysiol.* **26**, 403–415.