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ACTIVATION AND INHIBITION OF OLFACTORY BULB NEURONES BY ANTERIOR COMMISSURE VOLLEYS IN THE RABBIT

BY KENSAKU MORI AND SADAYUKI F. TAKAGI

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

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SUMMARY

1. In the rabbit olfactory bulb, analysis has been carried out on intracellular potentials recorded from mitral cells and neurones in the granule cell layer (g.c.l. cells) in addition to the extracellular field potentials in the olfactory bulb elicited by anterior commissure (a.c.) stimulation.

2. Most mitral cell recordings showed i.p.s.p.s with latency of 7–11 msec following a.c. stimulation. These i.p.s.p.s were similar to those evoked by lateral olfactory tract (l.o.t.) stimulation in their sensitivity to internally applied current and showed asymmetrical reversal during application of the hyperpolarizing current.

3. Volleys in the a.c. elicited e.p.s.p.s in type 1 g.c.l. cells whose characteristics were in agreement with those of inhibitory interneurones inferred from the analyses of mitral cell i.p.s.p.s. It has been suggested that these type 1 g.c.l. cells may be the common inhibitory interneurones (presumably granule cells) mediating both a.c.-evoked and l.o.t.-evoked i.p.s.p.s in mitral cells.

4. Conditioning a.c. stimulation depressed the test l.o.t.-evoked i.p.s.p.s in mitral cells and test l.o.t.-evoked e.p.s.p.s in type 1 g.c.l. cells. These observations are in good agreement with the hypothesis that l.o.t.-evoked i.p.s.p.s are mainly mediated by the dendrodendritic reciprocal synapses between mitral cell dendrites and peripheral processes of granule cells.

5. The results are discussed in relation to the inhibitory mechanisms controlling mitral cell activity in the olfactory bulb.

INTRODUCTION

In the preceding paper (Mori & Takagi, 1978), it was shown that lateral olfactory tract (l.o.t.) stimulation activated presumed granule cells in the olfactory bulb through mitral-to-granule dendrodendritic excitatory synapses and that mitral cell i.p.s.p.s seemed to be mediated by these granule cells. Since the inhibition of the mitral cell activity can be produced not only by stimulation of l.o.t. but also by stimulation of anterior commissure (a.c.) (Baumgarten, Green & Mancia, 1962; Ochi, 1963; Yamamoto, Yamamoto & Iwama, 1963), it was desirable to extend the previous study of the inhibitory mechanisms controlling mitral cell activity by analysing the responses of olfactory bulb neurones to a.c. stimulation. In the early part of the present paper, intracellularly recorded responses of mitral cells and of neurones in the granule cell layer (g.c.l. cells) to a.c. stimulation will be described

and they will be compared to their responses to l.o.t. stimulation. In the later part, the effect of the a.c. stimulation upon the l.o.t.-evoked e.p.s.p.s in g.c.l. cells and l.o.t.-evoked i.p.s.p.s in mitral cells will be described. The results presented here support the hypothesis that the granule cells which receive excitatory inputs from mitral cell dendrites are also activated by the a.c. stimulation through axo-dendritic or axo-somatic excitatory synapses located on the deep dendrites and soma of granule cells. The activated granule cells in turn inhibit the mitral cell activity through the granule-to-mitral dendrodendritic inhibitory synapses (*cf.* Shepherd, 1972). A preliminary account of part of this work has been reported elswhere (Mori & Takagi, 1977*a*, *b*).

METHODS

Twenty-nine albino rabbits weighing between 1·7 and 3 kg were used. The general experimental procedures were the same as those described in the preceding paper (Mori & Takagi, 1978). In this experiment, bipolar electrodes of acupuncture needles insulated except the tips were used to stimulate the anterior commissure. These electrodes were inserted into the anterior commissure in the contralateral forebrain about 1 mm lateral from the midline. The final position of the electrode tips was such as to obtain the maximum a.c.-evoked field potential in the g.c.l. of the olfactory bulb. A small electrolytic lesion was made at the site of stimulation at the end of the experiment and the location of the electrode tip was subsequently checked histologically. The unitary and potential wave responses recorded in the olfactory bulb to a.c. stimulation ceased completely after sectioning the a.c. at the midline or in the ipsilateral forebrain caudal to the anterior olfactory nucleus.

RESULTS

Field potentials in the olfactory bulb elicited by a.c. stimulation

It has been demonstrated by several authors that stimulation of the a.c. elicits a large negative-going potential in the g.c.l. of the olfactory bulb (Kerr, 1960; Ochi, 1963; Callens, 1965; Dennis & Kerr, 1968; Nicoll, 1970). Nicoll (1970) showed that this negative going potential reverses its polarity near the mitral cell layer (m.c.l.) and becomes a smaller positive wave in the external plexiform layer. In the first step to analyse the responses of olfactory bulb neurones to a.c. stimulation, it was important to reinvestigate the depth profile of the a.c.-evoked field potential in the olfactory bulb and to compare it with the depth profile of the l.o.t.-evoked field potential because the latter has been analysed most extensively by several investigators (Phillips, Powell & Shepherd, 1963; Rall, Shepherd, Reese & Brightman, 1966; Rall & Shepherd, 1968; Nicoll, 1969).

Fig. 1*B* shows the a.c.-evoked field potentials recorded at various depths in the olfactory bulb. In this series of experiments, the field potentials evoked by l.o.t. stimulation were also recorded at the same depths as above (Fig. 1*A*). The amplitudes of the potentials were measured at the point of 6 msec after the l.o.t. stimulation in column *A* (which is near the peak of component 2 of the field potential, *cf.* Mori & Takagi, 1978) and 25 msec after a.c. stimulation in column *B*. The depth profiles are shown graphically in Fig. 1*C*. As is well known (e.g. Rall & Shepherd, 1968), component 2 of the field potential evoked by l.o.t. stimulation is a large positivity in the middle area of the g.c.l. (*g–j* in Fig. 1*A*) and a large negativity in the external plexiform layer (e.p.l.) (*b–d* in Fig. 1*A*). Thus, there is a flow of extracellular current from the g.c.l. to e.p.l. Rall & Shepherd (1968) showed that this current flow could be

reconstructed by assuming that there is a strong membrane depolarization of the peripheral processes of granule cells in the e.p.l. The negativity in the e.p.l. is due to this depolarization and the positivity in the g.c.l. is due to the extracellular current flow from deep dendrites or cell bodies of granule cells to the depolarized peripheral processes of granule cells. On the other hand, the a.c.-evoked field potential was a large negativity in the middle area of the g.c.l. (*g–j* in Fig. 1*B*). It reversed its polarity at the m.c.l. and became a positive wave in the e.p.l. (*b–d* in Fig. 1*B*).

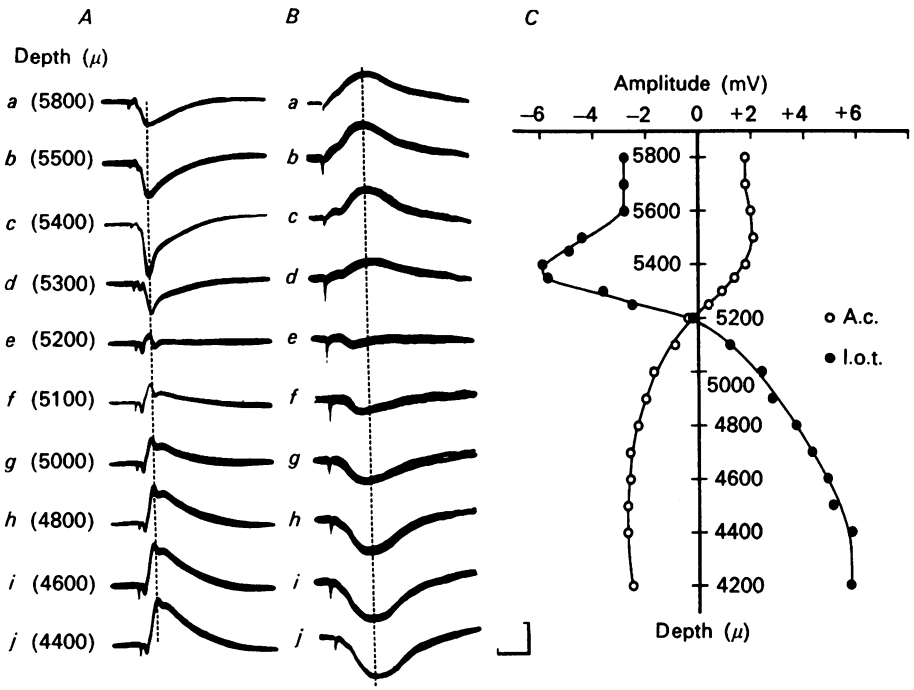

Fig. 1. A comparison of the field potentials in the olfactory bulb evoked by l.o.t. and a.c. stimulation. *A*, field potentials evoked by l.o.t. stimulation were recorded at varying depths; the amplitudes of the field potentials (component 2) as measured at a latency of 6 msec (vertical broken line) are plotted against depth in *C* (filled circles). *B*, field potentials elicited by a.c. stimulation, the amplitudes of the field potentials were measured at a latency of 20 msec (vertical broken line), which are also plotted against depth in *C* (open circles); the depth of the recording electrode in the olfactory bulb is indicated at the left of each row; the records were obtained from the ventral side of the olfactory bulb. Vertical bar: 4 mV for *A*; 2 mV for *B*. Horizontal bar: 10 msec for *A*; 20 msec for *B*. Positivity is upwards.

This indicates a flow of extracellular current with opposite direction to that caused by l.o.t. stimulation: a current flow from the e.p.l. to g.c.l. These observations suggest that the flow of extracellular current caused by a.c. stimulation may be mainly produced by the depolarization of deep dendrites or cell bodies of granule cells in the g.c.l.; the negativity in the g.c.l. may be due to this depolarization while the positivity in the e.p.l. may be due to the corresponding current source.

Responses of mitral cells to a.c. stimulation

Volleys in the anterior commissure cause a hyperpolarization of the mitral cell membrane (Yamamoto *et al.* 1963). Fig. 2*A*, *a*, *b* and *c* demonstrates such membrane hyperpolarizations of a cell located at the m.c.l. which were elicited by a.c. stimulation with one, two and three shocks respectively. This cell was identified as a

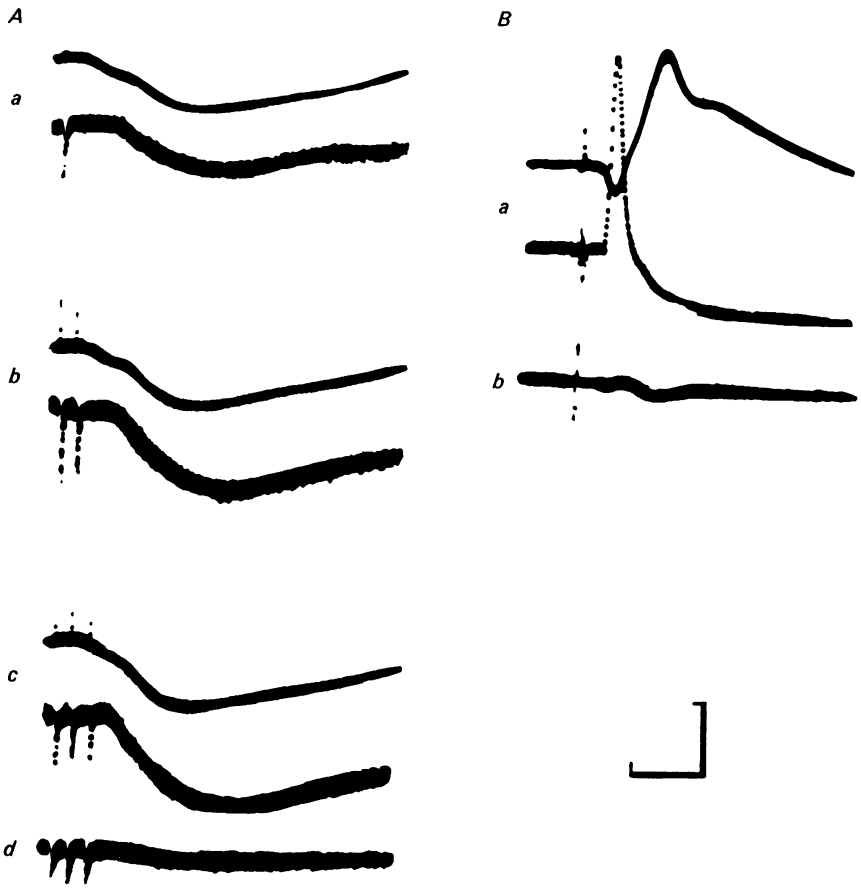

Fig. 2. Intracellular potentials of a mitral cell evoked by a.c. and l.o.t. stimulation. *A*, mitral cell responses to a.c. stimulation (lower traces of *a*, *b* and *c*) together with the simultaneously recorded field potential in the g.c.l. (upper traces); in *a*, *b* and *c*, a.c. was stimulated once, twice and three times respectively. *B*, the response to l.o.t. stimulation (lower trace of *a*) obtained from the same mitral cell as *A*; the upper trace of *a* shows the simultaneously recorded field potential in the g.c.l. The extracellular field potentials recorded just after withdrawal are shown in *A*, *d* and *B*, *b*. Vertical bar: 4 mV for upper traces of *A*, *a*, *b*, *c* and *B*, *a*; 10 mV for lower traces of *A*, *a*, *b*, *c* and *d*; 20 mV for lower trace of *B*, *a* and *b*. Horizontal bar: 10 msec for *A*; 4 msec for *B*.

mitral cell by antidromic activation through the l.o.t. (Fig. 2*B*). The latency of the hyperpolarization evoked by a single a.c. volley was 8 msec. In other cells, this latency ranged from 7 to 11 msec. The amplitude of the hyperpolarization increased when the additional volleys were applied at short intervals (Fig. 2*A*, *b* and *c*). In

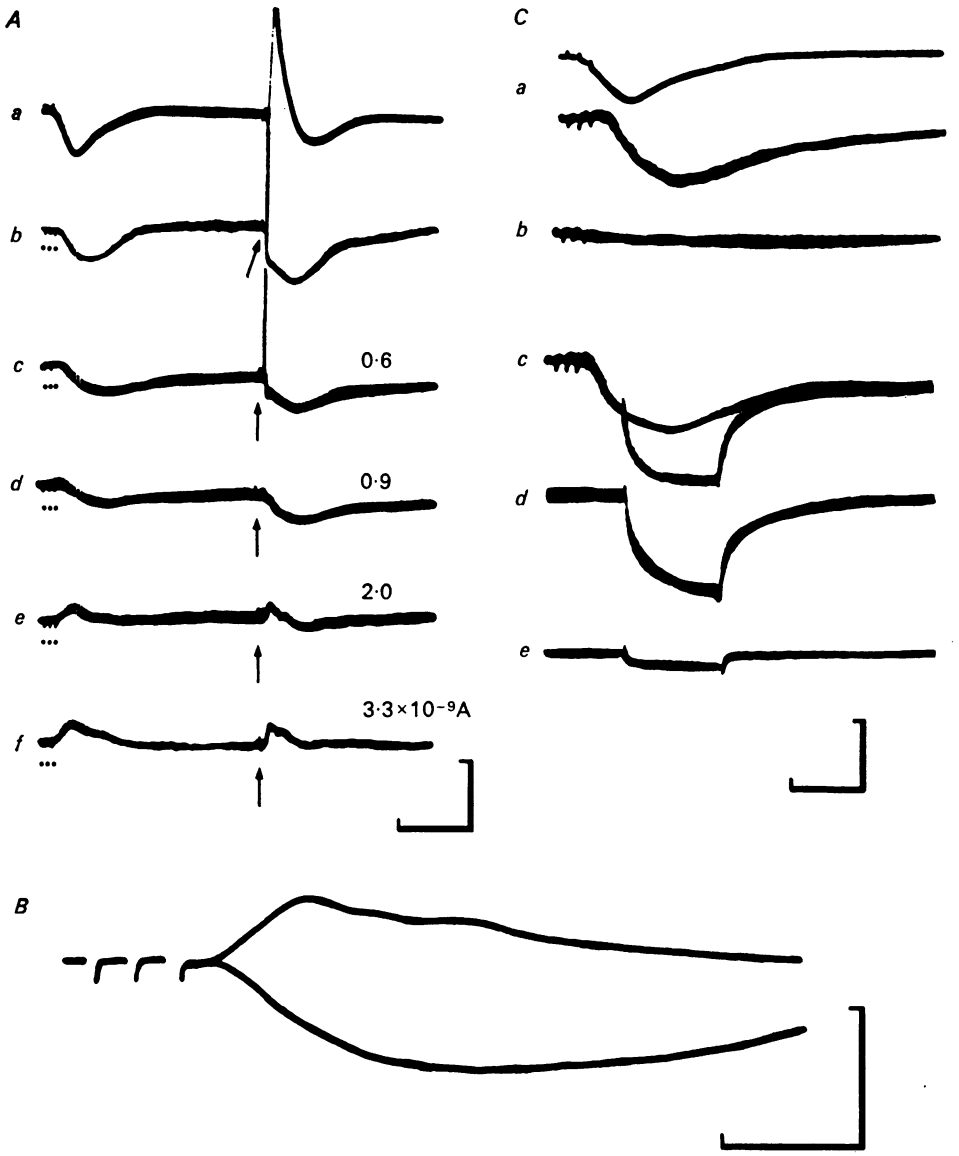

Fig. 3. Intracellular records of i.p.s.p.s from mitral cells. In *A*, both a.c. (three dots) and l.o.t. (arrows) were stimulated with an interval of 120 msec in one sweep; trace *a* shows the field potential recorded in the middle area of the g.c.l.; the intracellular potentials from a mitral cell are shown in *b–f*; in *c–f*, hyperpolarizing currents were applied intracellularly; the amount of the applied currents is indicated on the right shoulder of each trace. *B* shows the superimposed tracings of the original hyperpolarizing i.p.s.p.s elicited by anterior commissure stimulation and the reversed i.p.s.p.s caused by the internal application of the hyperpolarizing currents ($3{\cdot}0 \times 10^{-9}$ A). *C*, i.p.s.p.s of another mitral cell elicited by a.c. stimulation (lower trace of *a*) are shown together with the simultaneously recorded field potential in the g.c.l. (upper trace of *a*); the trace in *b* is the field potential recorded just after withdrawal of the micro-electrode. In *C*, a hyperpolarizing current pulse of 9×10^{-10} A was sometimes applied through the recording electrode during the i.p.s.p.; control intracellular and extracellular voltage drop produced by the same current pulse without conditioning a.c. stimulation are shown in *d* and *e* respectively. Vertical bar in *A*: 4 mV for *a* and 20 mV for *b–f*. Horizontal bar in *A*: 40 msec. Vertical bar in *B*: 10 mV. Horizontal bar in *B*: 10 msec. Vertical bar in *C*: 4 mV for upper trace of *a*, 10 mV for lower trace of *a* and *b–e*. Horizontal bar in *C*: 20 msec. Spikes retouched.

some instances, double shocks evoked hyperpolarizations in mitral cells even when the stimulus strength was weakened so that a single a.c. stimulation failed to elicit them. When responses to strong a.c. volleys (three shocks with 30 volts and 0·1 msec duration) were examined in fifty-five antidromically identified mitral cells, all the mitral cells showed the hyperpolarization. The resting membrane potential of these mitral cells ranged from -45 to -70 mV. As shown in Fig. 3*A*, the amplitude of the a.c.-evoked hyperpolarization was decreased and then reversed in polarity when increasing hyperpolarizing currents were applied intracellularly. This observation indicates that this a.c.-evoked hyperpolarization is the i.p.s.p. produced by the inhibitory synapses on the mitral cell. From the fact that the onset latencies of the i.p.s.p.s evoked in the mitral cells by a.c. stimulation were relatively long compared with those of the extracellular field potentials recorded in the g.c.l., and that a single weak shock to a.c. was sometimes ineffective in producing the i.p.s.p.s, it may be postulated that the i.p.s.p.s in the mitral cells are not produced monosynaptically by a.c. stimulation, but there may be inhibitory interneurones responsible for the a.c.-evoked i.p.s.p.s in the mitral cells. It can also be seen in Fig. 2*A* and *B* that the a.c.-evoked i.p.s.p.s have a much slower rising phase than the l.o.t.-evoked i.p.s.p.s in mitral cells.

In Fig. 3*A* (*b–f*) are shown the comparison of the effects of internally applied currents upon the a.c.-evoked i.p.s.p. and the l.o.t.-evoked i.p.s.p. in a mitral cell. In this figure the trace in *b* shows a response of a mitral cell to a.c. stimulation (indicated by three dots) and then a response to l.o.t. stimulation (indicated by the upward arrow). When a hyperpolarizing current was applied intracellularly, the amplitude of the a.c.-evoked i.p.s.p. decreased in parallel with the amplitude of the l.o.t.-evoked i.p.s.p. (*c* and *d*). Further increase of the hyperpolarizing current reversed both i.p.s.p.s into depolarizing responses (*e* and *f*). In *e* it can be seen that the reversal of the a.c.-evoked i.p.s.p. could be produced by almost the same amount of current required for reversal of the l.o.t.-evoked i.p.s.p. Furthermore, the reversed i.p.s.p.s elicited by a.c. volleys during application of hyperpolarizing current were quite asymmetrical in shape with the original hyperpolarizing i.p.s.p. (Fig. 3*B*) as is the case in the l.o.t.-evoked i.p.s.p.s (Mori & Takagi, 1978). These observations suggest that the inhibitory synapses responsible for the a.c.-evoked i.p.s.p. are also distributed widely on the soma and dendrites of the mitral cell. Fig. 3*C*, *a* shows the i.p.s.p. recorded in another mitral cell following a.c. stimulation (lower trace) and simultaneously recorded field potential in the g.c.l. (upper trace). During this i.p.s.p., a hyperpolarizing current pulse of 0·9 nA was applied intracellularly (Fig. 3*C*, *c*). When the voltage drop produced by this hyperpolarizing current pulse was compared with that produced by the same current pulse without conditioning a.c. stimulation (Fig. 3*C*, *d*), the former had a smaller amplitude and a faster time course than the latter. This indicates that the i.p.s.p.s of mitral cells caused by a.c. stimulation were accompanied by an increase in the conductance of the mitral cell membrane.

Responses of granule layer cells to a.c. stimulation

In the preceding paper, (Mori & Takagi, 1978) it has been shown that type 1 g.c.l. cells (presumably granule cells) receive an excitatory input from mitral cell dendrites. In addition to this, the analysis of the field potentials elicited by a.c.

stimulation and of the a.c.-evoked i.p.s.p.s in mitral cells suggests that granule cells may receive an excitatory input from the a.c. It therefore seemed important to record the responses of type 1 g.c.l. cells to a.c. volleys in order to elucidate whether or not there is convergence on the same type 1 g.c.l. cells of excitatory inputs from both the mitral cell dendrites and the a.c. Fig. 4*C* shows an e.p.s.p. in a type 1 g.c.l. cell elicited by l.o.t. stimulation. This cell was classified as a type 1 g.c.l. cell because e.p.s.p.s in this cell evoked by test l.o.t. stimulation were markedly depressed

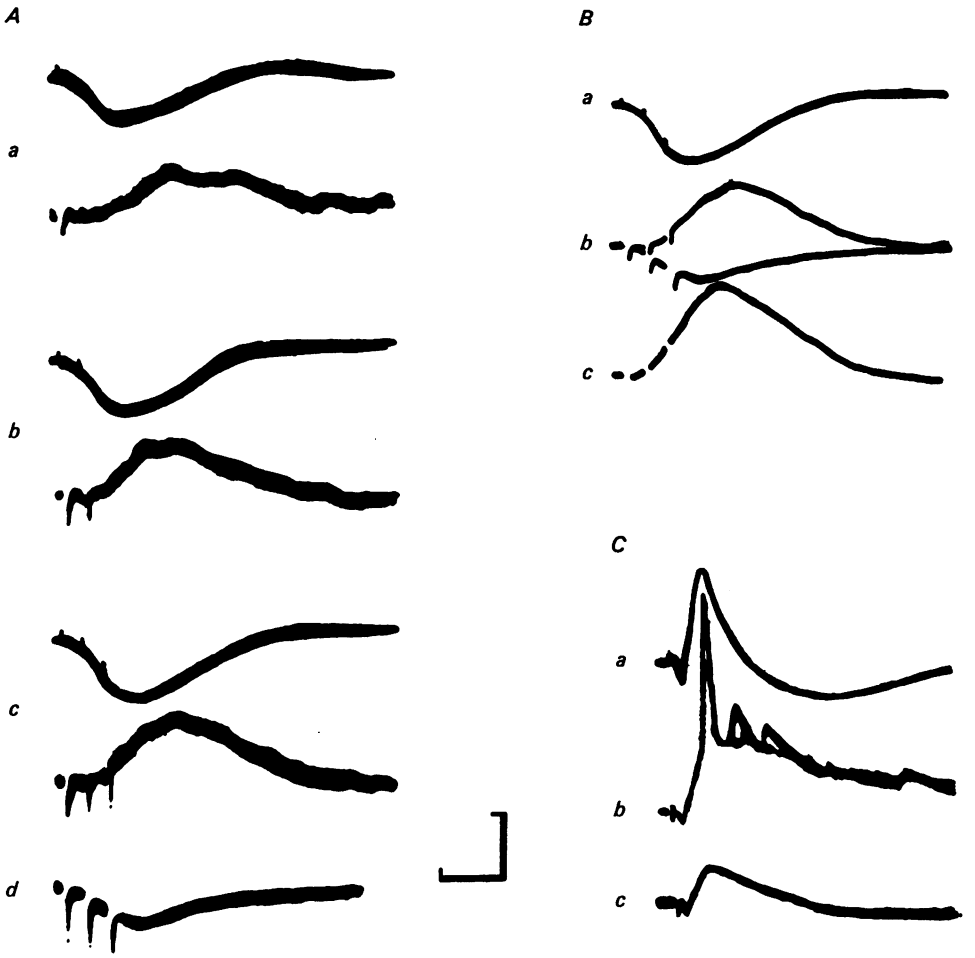

Fig. 4. Responses of g.c.l. cells to a.c. stimulation. *A*, intracellular potentials from a g.c.l. cell (lower traces in *a–c*) and simultaneously recorded field potentials in the g.c.l. (upper trace in *a–c*); in *d* is shown the field potential recorded just outside the recorded g.c.l. cell. *B*, *a* is the field potential in the middle area of g.c.l.; superimposed tracings of the intracellular potential from a g.c.l. cell (upper trace) and just extracellular potential (lower trace) are shown in *b*; the true membrane potential (*c*) was obtained by subtracting the just extracellular potential from the intracellular potential. *C*, response of the same g.c.l. cell as *A* to l.o.t. stimulation (*b*) together with the field potential in g.c.l. (*a*); extracellular field potential recorded just after withdrawal is shown in *c*. Vertical bar: 4 mV for upper traces of *A*, *a–c*, *B*, *a* and *C*, *a*: 10 mV for the other traces. Horizontal bar: 20 msec for *A* and *B*; 10 msec for *C*. Spikes retouched.

by the conditioning l.o.t. stimulation (*cf.* Mori & Takagi, 1978). When a single volley was applied to the a.c., this cell showed an e.p.s.p. with onset latency of about 5 msec. This e.p.s.p. increased in amplitude when additional shocks were applied to a.c. at short intervals (Fig. 4*A*, *b* and *c*). Since the extracellular field potential just outside the impaled cell was large, the time course of the true membrane potential was determined by subtracting the extracellular field potential from the intracellularly recorded potential (Fig. 4*B*). It may be noted in Fig. 4*B* (and also in Fig. 6*B*) that the onset time and duration of the a.c.-evoked e.p.s.p. correspond with those of the negative wave of the g.c.l. field potential. The responses to a.c. stimulation were examined in thirty type 1 g.c.l. cells with resting membrane potential

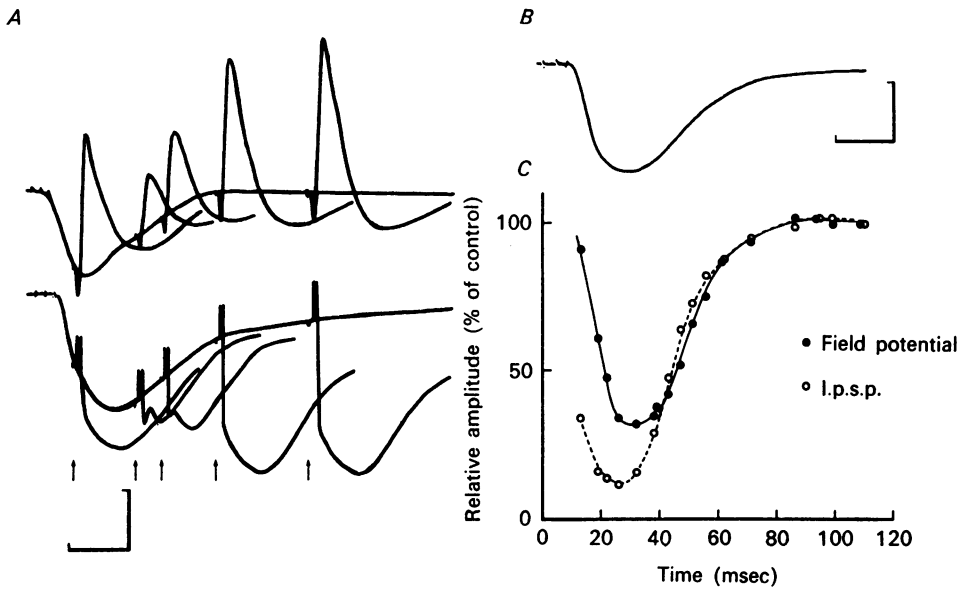

Fig. 5. The effect of conditioning a.c. stimulation on mitral cell i.p.s.p.s evoked by test l.o.t. stimulation. *A*, superimposed tracings of the intracellular records from a mitral cell (lower traces) and the field potentials in the g.c.l. (upper traces); the times of the test l.o.t. stimulation were indicated by arrows. *B*, the i.p.s.p. elicited by the conditioning a.c. stimulation is shown with the same time scale as in *C*. *C*, plot of relative amplitudes of the mitral cell 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 *A*. Calibrations: *A*, vertical bar: 2 mV for upper traces, 5 mV for lower traces; horizontal bar: 20 msec; *B*, vertical bar: 5 mV; horizontal bar: 20 msec.

of -40 to -64 mV and clear e.p.s.p.s were seen in twenty-seven cells. Thus, most of the type 1 g.c.l. cells receive excitatory inputs both from mitral cell dendrites and from the a.c. It is interesting to note that a.c. stimulation failed to elicit spike activity in most of the type 1 g.c.l. cells which showed an e.p.s.p. with superimposed spikes following l.o.t. stimulation (Figs. 4 and 6).

Effects of conditioning a.c. stimulation upon the responses of mitral cells or g.c.l. cells to test l.o.t. stimulation

It was shown in the preceding paper (Mori & Takagi, 1978) that conditioning l.o.t. stimulation caused the depression of test l.o.t.-evoked e.p.s.p.s in type 1 g.c.l. cells and test l.o.t.-evoked i.p.s.p.s in mitral cells. These results were ascribed to the blocking of antidromic activation of mitral cell somata and dendrites by the preceding i.p.s.p.s in mitral cells which were elicited by the conditioning l.o.t. stimulation. Since strong a.c. stimulation causes large i.p.s.p.s in mitral cells (*cf.* Fig. 2), they may also block the antidromic activation of mitral cell somata and dendrites following l.o.t. stimulation. Thus, one might expect that conditioning a.c. stimulation also depresses the test l.o.t.-evoked e.p.s.p.s in type 1 g.c.l. cells and test l.o.t.-evoked i.p.s.p.s in mitral cells, if the dendrodendritic pathways should be responsible for activation of the type 1 g.c.l. cells and the subsequent inhibition of mitral cells. Fig. 5*A* shows the superimposed tracings of intracellular records from a mitral cell (lower traces) together with the records of the simultaneously elicited field potentials in the middle area of g.c.l. (upper traces). Conditioning a.c. stimulation (three shocks in the extreme left) caused i.p.s.p.s in the mitral cell and a negative-going extracellular potential in the g.c.l. Following the conditioning a.c. stimulation, the responses of this cell to test l.o.t. stimulation were examined at various conditioning-test intervals. It can be seen in *A* that there was a clear depression of i.p.s.p.s in the mitral cell and of the field potentials in the g.c.l. evoked by the test l.o.t. stimulation. In *C*, the relative amplitudes of the conditioned test i.p.s.p.s in the mitral cell (open circles) and those of the test field potentials in the g.c.l. (filled circles) as percentages of their unconditioned control values were plotted against the conditioning-test interval. When the time course of the depressions of the test i.p.s.p.s in mitral cells and that of the test field potentials in g.c.l. were compared with that of the i.p.s.p. evoked by a conditioning a.c. stimulation, clear correlation could be seen between them (Fig. 5*B* and *C*): the depressions of the test i.p.s.p.s in mitral cells and test field potentials in the g.c.l. were maximal when the time of the test l.o.t. stimulation coincided with the peak of the i.p.s.p. evoked by a conditioning a.c. stimulation. Such a depression of the test i.p.s.p.s by a conditioning a.c. stimulation was observed in most of the impaled mitral cells (twenty-two out of twenty-four cells examined).

Fig. 6 shows an intracellular recording of a type 1 g.c.l. cell together with the recording of the simultaneously elicited field potentials in the middle area of the g.c.l. This cell showed a large e.p.s.p. with superimposed spikes following l.o.t. stimulation (Fig. 6*A*, *b*) and a.c. stimulation also caused a large e.p.s.p. (Fig. 6*B*, *b*). Next, the effect of conditioning a.c. stimulation was studied upon the e.p.s.p.s caused by test l.o.t. stimulation (*C–H*). As the interval between a conditioning and a test stimulation was decreased, the test e.p.s.p.s were depressed markedly (*C–F*). However, when the conditioning-test intervals were further decreased, as in Fig. 6*G* and *H*, the test e.p.s.p.s were restored and summated with the e.p.s.p. elicited by the conditioning a.c. stimulation. In Fig. 7*B*, the relative amplitudes of the e.p.s.p.s and the field potentials in the g.c.l. elicited by the test l.o.t. stimulation are shown as percentages of their unconditioned control values against the conditioning-test

interval. It can be seen in this Figure that the depression of test e.p.s.p.s in type 1 g.c.l. neurones has similar time course as that of the test field potential in the g.c.l. One of the possible explanations for this depression of the test l.o.t.-evoked e.p.s.p.s by the conditioning a.c. stimulation may be that it is caused by an increase in membrane conductance of the g.c.l. neurones due to i.p.s.p.s elicited by the conditioning a.c. stimulation (*cf.* Yamamoto *et al.* 1963). In fact, a.c. stimulation sometimes elicited a hyperpolarization following large e.p.s.p.s in the type 1 g.c.l. cells (Fig. 6*B*, *b* and Fig. 7*A*, *a*). However, the time course of the depression in Fig. 7*B* does

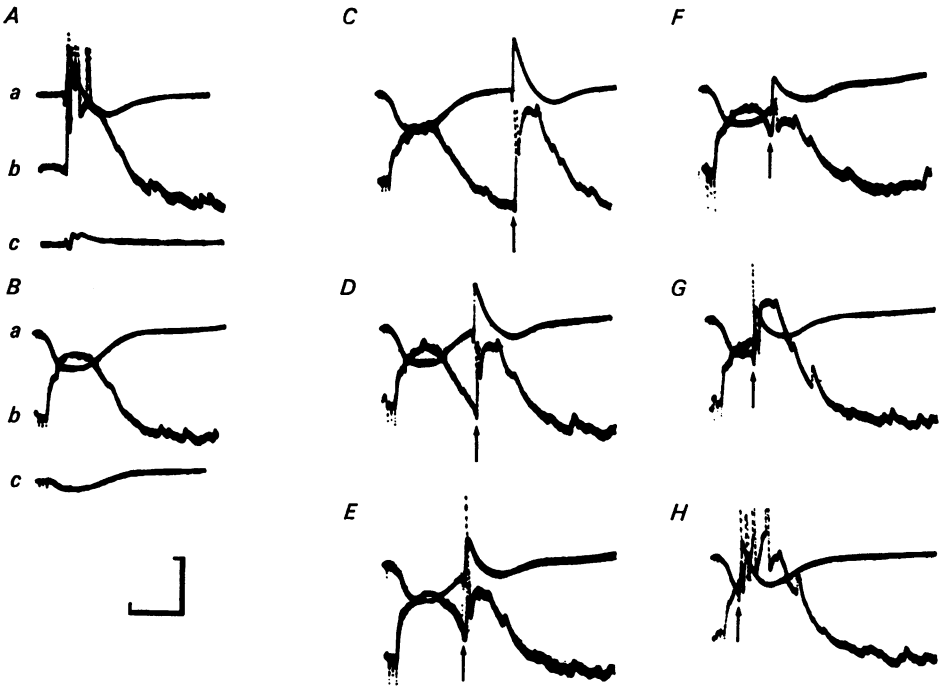

Fig. 6. The effect of conditioning a.c. stimulation on e.p.s.p.s in a g.c.l. cell evoked by test l.o.t. stimulation. *A*, intracellularly recorded response of a g.c.l. cell to l.o.t. stimulation (*b*) and simultaneously recorded field potential in the middle area of the g.c.l. (*a*). *B*, intracellularly recorded response of the same g.c.l. cell to a.c. stimulation (*b*) and the field potential in the g.c.l. (*a*). *A*, *c* and *B*, *c* show the extracellular field potential recorded just outside of the impaled g.c.l. cell. *C–H*, suppression of the test l.o.t.-evoked e.p.s.p.s in the g.c.l. cell (lower traces) and that of the test field potential in the g.c.l. (upper traces) by the conditioning a.c. stimulation. The time of test l.o.t. stimulation was indicated by an upward arrow in each trace. Vertical bar: 4 mV for field potentials in the g.c.l. (upper traces); 10 mV for intracellular potentials (*A*, *b*, *B*, *b* and lower traces in *C–H*) and just extracellular potentials (*A*, *c* and *B*, *c*). Horizontal bar: 40 msec. Spikes retouched.

not follow this hyperpolarization (compare Fig. 7*A*, *a* and Fig. 7*B*). Furthermore, strong conditioning a.c. stimulation nearly completely depressed the test l.o.t.-evoked e.p.s.p.s in type 1 g.c.l. cells (Mori & Takagi, 1977*a*). This observation cannot be explained by a conductance increase of the post-synaptic membrane because there would have to be infinite conductance in order to depress the test l.o.t.-evoked

e.p.s.p.s completely. On the other hand, when the plot of the depression of the test l.o.t.-evoked e.p.s.p.s (Fig. 7*B*) and the shape of the a.c.-evoked i.p.s.p.s in mitral cells are compared, it can be seen that they have similar time courses. For example, in the case of Fig. 7*B*, the amplitudes of both test l.o.t.-evoked e.p.s.p.s in the g.c.l. cell and the test field potential in the g.c.l. decreased maximally with a conditioning-

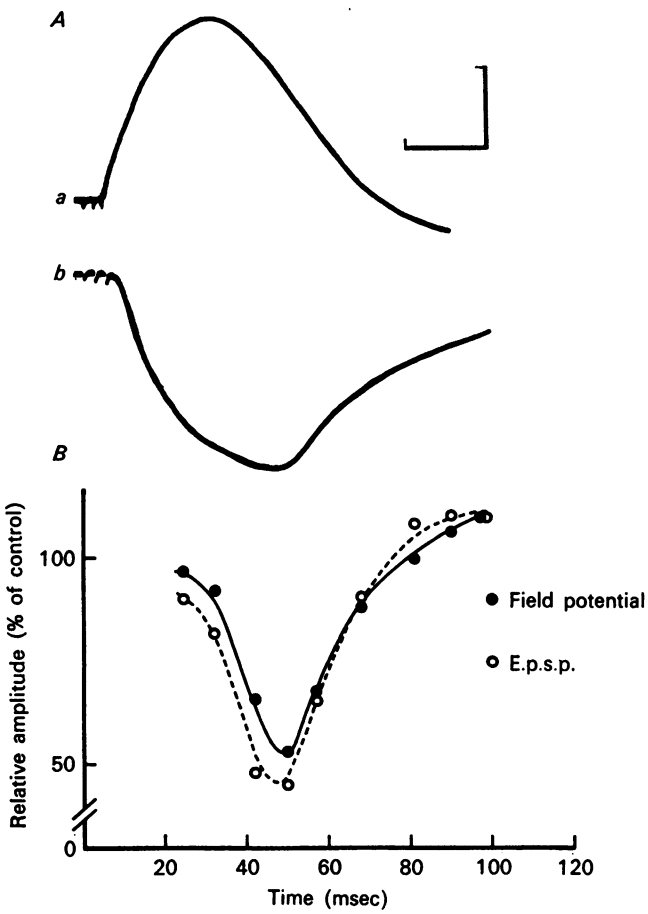

Fig. 7. The time course of the depression of the test l.o.t.-evoked e.p.s.p.s in a g.c.l. cell and the test l.o.t.-evoked field potential in the g.c.l. after a conditioning a.c. stimulation. *A*, an intracellular response of the same g.c.l. cell as in Fig. 6 (*a*) and that of a mitral cell (*b*) to a.c. stimulation with the same time scale as in *B*; both of them were recorded in the course of a single micro-electrode penetration. *B*, relative amplitudes of the e.p.s.p.s in the same g.c.l. cell as in Fig. 6 (open circles) and the field potentials in the g.c.l. (filled circles) evoked by the test l.o.t. stimulation after a conditioning a.c. stimulation are calculated and shown as % of their unconditioned control values (ordinate). The interval between the conditioning and the test stimulations is shown in abscissa. Vertical bar: 5 mV. Horizontal bar: 20 msec.

test interval of 40–60 msec. At this time, the i.p.s.p.s elicited in many mitral cells by a.c. stimulation were also maximal in amplitude, if the stimulus intensity was equal to that used for the conditioning a.c. stimulation in Fig. 7*B*. Though such a comparison was obtained in only five cases, the time course of the depression of

test l.o.t.-evoked e.p.s.p.s in type 1 g.c.l. cells was similar to that of the a.c.-evoked i.p.s.p.s in mitral cells in all cases. These results are in good agreement with the hypothesis that i.p.s.p.s in mitral cells elicited by conditioning a.c. stimulation block the antidromic activation of mitral cell somata or dendrites and thereby prevent the activation of mitral-to-granule dendrodendritic excitatory synapses, resulting in the depression of test l.o.t.-evoked e.p.s.p.s in granule cells and test l.o.t.-evoked i.p.s.p.s in mitral cells (*cf.* Nicoll, 1969).

DISCUSSION

Properties of i.p.s.p.s in mitral cells elicited by a.c. stimulation

In nearly all mitral cell recordings, stimulation of the a.c. showed (possibly disynaptic and polysynaptic) i.p.s.p.s. These i.p.s.p.s showed the following characteristics as compared with l.o.t.-evoked i.p.s.p.s.

I. The a.c.-evoked i.p.s.p.s had a much slower rising phase than the l.o.t.-evoked i.p.s.p.s.

II. Conditioning l.o.t. stimulation had little effect on the test a.c.-evoked i.p.s.p.s, while it markedly depressed the test l.o.t.-evoked i.p.s.p.s.

III. Paired or repetitive a.c. stimulation augmented the amplitudes of a.c.-evoked i.p.s.p.s, while l.o.t.-evoked i.p.s.p.s were usually depressed by the preceding strong a.c. stimulation.

From electronmicroscopic observations that almost all synapses found on mitral cells (except in or around the glomerular region) are reciprocal synapses with peripheral processes of granule cells (Price & Powell, 1970*c*; Reese & Shepherd, 1972) and that a.c. fibres terminate on the granule cells (Price & Powell, 1970*b*), one can assume that granule cells are the inhibitory interneurones responsible for the a.c.-evoked i.p.s.p.s in mitral cells. If this is true, it is important to elucidate whether the granule cells are common interneurones responsible for both a.c.-evoked i.p.s.p.s and l.o.t.-evoked i.p.s.p.s in mitral cells, or if there are two subgroups of granule cells, one mediating the a.c.-evoked i.p.s.p., the other mediating the l.o.t.-evoked i.p.s.p. One of the methods to test these alternatives was to compare the sensitivity of the a.c.-evoked i.p.s.p.s to internally applied current with that of l.o.t.-evoked i.p.s.p.s. If the locations of inhibitory synapses responsible for a.c.-evoked i.p.s.p.s on the mitral cell are quite different from those for l.o.t. evoked i.p.s.p.s, then a.c.-evoked i.p.s.p.s and l.o.t.-evoked i.p.s.p.s would be expected to show different sensitivity to the internally applied current. The a.c.-evoked i.p.s.p.s in mitral cells, however, had similar sensitivity as the l.o.t.-evoked i.p.s.p.s to the internally applied current; the reversal of both of the i.p.s.p.s occurred at the same time during passage of the progressively increasing hyperpolarizing current. Furthermore, a.c.-evoked i.p.s.p.s showed quite asymmetrical reversal during application of hyperpolarizing current, suggesting that the inhibitory synapses responsible for the a.c.-evoked i.p.s.p.s are also distributed widely on mitral cell soma and dendrites, just as those responsible for l.o.t.-evoked i.p.s.p.s. These results suggest that the granule cells may be common inhibitory interneurones mediating both a.c.-evoked i.p.s.p.s and l.o.t.-evoked i.p.s.p.s in mitral cells. If this hypothesis is correct, one may expect a penetrating micro-electrode to encounter interneurones in the granule cell layer,

which have the following characteristics. These points were inferred from the studies of the a.c.-evoked i.p.s.p.s and l.o.t.-evoked i.p.s.p.s in mitral cells:

I. Monosynaptic excitation from l.o.t.

II. Excitation by a volley in a.c.

III. The a.c.-evoked e.p.s.p.s have a much slower rising phase than the l.o.t.-evoked e.p.s.p.s.

IV Conditioning l.o.t. or a.c. stimulation markedly depresses the test l.o.t.-evoked e.p.s.p.s.

V. Paired or repetitive a.c. stimulation augments the amplitude of the e.p.s.p.s.

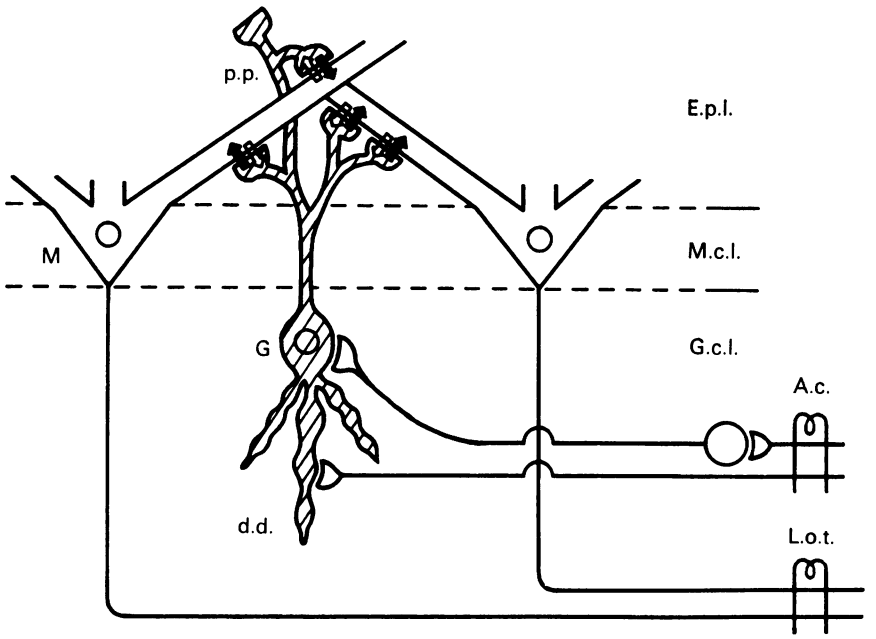

Fig. 8. Schematic diagram of the synaptic pathways in the deep layers of the olfactory bulb. E.p.l.: external plexiform layer; M.c.l.: mitral cell layer; G.c.l.: granule cell layer. M: mitral cell; G: granule cell; p.p.: peripheral process of granule cell; d.d.: deep dendrite of granule cell; A.c.: anterior commissure; l.o.t. lateral olfactory tract.

Activation of type 1 g.c.l. cells by a.c. volley

The results reported here and in the preceding paper (Mori & Takagi, 1978) show that most of the type 1 g.c.l. cells exhibited all the properties listed above (*cf.* Fig. 4 and Fig. 6). For example, excitatory input to the type 1 g.c.l. cells from the anterior commissure was found in twenty-seven out of thirty type 1 g.c.l. cells. These results suggest that the type 1 g.c.l. cells may be the granule cells which mediate both l.o.t.-evoked i.p.s.p.s and a.c.-evoked i.p.s.p.s in mitral cells. Furthermore, the analysis of the field potential in the olfactory bulb elicited by a.c. stimulation indicates that an a.c. volley activates the excitatory synapses located on the deep dendrites and somata of granule cells. These conclusions are in good agreement with electron microscopic observations by Price & Powell (1970*b*), who found that the fibres of the anterior commissure terminate upon spines and varicosities of the deep dendrites and upon somatic spines of granule cells. Since the a.c.-evoked e.p.s.p.s in the type 1

g.c.l. cells are long lasting, it is possible that a.c. stimulation causes not only monosynaptic but also disynaptic (or polysynaptic) activation of granule cells. One of the possible interneurones may consist of neurones in the ipsilateral anterior olfactory nucleus because their axons enter the olfactory bulb and terminate upon the granule cells (Price & Powell, 1970*b*) and they were activated by a.c. stimulation (Mori, K. & Takagi, S. F., unpublished observations).

The neuronal pathway of mitral cell inhibition following a.c. or l.o.t. stimulation

The results presented here and in the preceding paper confirm the hypothesis proposed by Rall *et al.* (1966) and Rall & Shepherd (1968) that l.o.t.-evoked i.p.s.p.s in mitral cells are mainly mediated by dendrodendritic reciprocal synapses between mitral cell dendrites and peripheral processes of granule cells. In Fig. 8 is shown a schematic diagram of the neuronal pathways of l.o.t.-evoked and a.c.-evoked i.p.s.p.s in mitral cells, based largely on a comparison of the anatomical investigations (Cajal, 1955; Rall *et al.* 1966; Price & Powell, 1970*a*, *b*, *c*) and previous (Rall & Shepherd, 1968; Nicoll, 1969) and present electrophysiological investigations. According to this diagram, the pathway of mitral cell inhibition following l.o.t. or a.c. stimulation may be explained as follows. L.o.t. stimulation causes synchronous antidromic activation of mitral cell somata and dendrites and activates the peripheral processes of granule cells through the mitral-to-granule dendrodendritic excitatory synapses (open arrows). The activated peripheral processes in turn hyperpolarize the mitral cell dendrites through the granule-to-mitral dendrodendritic inhibitory synapses (closed arrows). On the other hand, a.c. stimulation causes monosynaptic e.p.s.p.s (and possibly disynaptic e.p.s.p.s via neurones in the anterior olfactory nucleus) in the deep dendrites and somata of granule cells. The depolarization of these sites in the granule cells causes negative field potential in the g.c.l. (Fig. 1*B*). The activated granule cells then inhibit mitral cell activity through the granule-to-mitral dendrodendritic inhibitory synapses (filled arrows). Since conditioning l.o.t. or a.c. stimulation causes i.p.s.p.s in mitral cells, test l.o.t. stimulation would fail to activate the somata and dendrites of the mitral cells. Thus, test l.o.t.-evoked e.p.s.p.s in granule cells would be depressed by the conditioning l.o.t. or a.c. stimulation (Fig. 6). Furthermore, the depression of the test l.o.t.-evoked e.p.s.p.s in granule cells should have similar time courses as those of the i.p.s.p.s elicited in the mitral cells by the conditioning stimulation, because the number of mitral cells which are activated antidromically by a test l.o.t. volley would be inversely proportional to the amplitude of i.p.s.p.s in the mitral cells caused by the conditioning volley. These explanations are in good agreement with the results reported here and in the preceding paper.

Functional significance

The present study provides evidence for the convergence of excitatory inputs from the mitral cell dendrites and from the anterior commissure on a common inhibitory interneurone (presumably granule cell). Thus, the activities of granule cells may be regulated not only by the input from the mitral cell dendrites but also by that from the anterior commissure. This indicates that the main relay neurones (mitral cells) in the olfactory bulb are influenced by impulses in anterior commissure

fibres indirectly via the granule cells. In fact, it has been reported that repetitive electrical stimulation of the anterior commissure inhibits the spontaneous discharges of mitral cells and the characteristic wave activity ('induced waves') in the olfactory bulb (Kerr & Hagbarth, 1955; Baumgarten *et al.* 1962; Yamamoto *et al.* 1963). It is interesting that the granule cells receive, in addition to the intrinsic synaptic input, various kinds of extrinsic fibre inputs; in addition to fibres from the anterior commissure are also centrifugal fibres running in the l.o.t. and fibres from the ipsilateral anterior olfactory nucleus (Price & Powell, 1970*b*). One of the possible functions of these extrinsic fibre inputs might be to modulate the dendrodendritic synaptic interactions between the mitral and granule cells. For example, when the granule cells are depolarized by a.c. fibres, there could be facilitation of transmission of the dendrodendritic negative feed-back pathways. Thus the mitral cell activity would cause more effective lateral- and self-inhibition of the mitral cells. On the other hand, when the granule cells are not depolarized, due to the absence of a.c. inputs, excitatory input from mitral cell dendrites might fail to activate a large number of granule-to-mitral dendrodendritic inhibitory synapses, resulting in more limited lateral or self-inhibition of the mitral cell activity. Though the functional significance for odour discrimination of these dendrodendritic interactions between mitral and granule cells is not deducible from experiments of the present type, further analysis of the synaptic interactions between them should contribute toward understanding the functional role of the olfactory bulb in processing of odour information in terms of its synaptic organization.

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