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ANALYSIS OF SYNAPTIC POTENTIALS IN MITRAL CELLS IN THE ISOLATED TURTLE OLFACTORY BULB

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SUMMARY

1. The synaptic responses of mitral cells have been analysed in intracellular recordings from the isolated olfactory bulb of the turtle.

2. The response of a mitral cell to a single volley in the lateral olfactory tract consisted of an antidromic impulse and a complex hyperpolarizing potential that had the properties of an inhibitory post-synaptic potential. The inhibitory response consisted of two successive components, I_1 and I_2, followed by a prolonged hyperpolarization.

3. High-gain recordings revealed miniature hyperpolarizing potentials during the I_1 and I_2 responses. Both the miniature potentials and the I_1 and I_2 responses were increased in amplitude by depolarizing injected currents, and decreased and reversed in polarity by hyperpolarizing currents. The input conductance was increased during the I_2 component. In some cells the I_1 and I_2 components, recorded with micropipettes filled with potassium acetate or potassium citrate, were depolarizing.

4. A single orthodromic volley in the olfactory nerves elicited a complex depolarizing-hyperpolarizing potential in mitral cells. The depolarization consisted of two successive components, E_1 and E_2. The hyperpolarization consisted of two successive components, I_1 and I_2, followed by a prolonged hyperpolarization.

5. The depolarizing components had the properties of excitatory post-synaptic potentials. They decreased in amplitude with depolarizing current injection and increased with hyperpolarizing injection. The hyperpolarizing components resembled the I_1 and I_2 components of the tract-evoked responses in their timing and properties.

6. It is postulated that the E_1 component reflects the initial excitation by olfactory nerve terminals of the mitral cell dendritic tufts in the olfactory glomeruli. The I_1 component is postulated to arise from dendrodendritic synaptic input mediated by interneurones, mainly granule cells. The E_2 and I_2 components are likely to arise mainly from intrinsic synaptic circuits within the olfactory bulb.

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INTRODUCTION

Mitral cells in the olfactory bulb are subjected to powerful inhibition following a single antidromic volley in the mitral cell axons in the lateral olfactory tract, or a single orthodromic volley in the olfactory receptor cell axons in the olfactory nerves. A variety of evidence has suggested that the pathways for this inhibition involve dendrodendritic reciprocal synapses between mitral cells and the granule cell interneurones (Rall, Shepherd, Reese & Brightman, 1966; Rall & Shepherd, 1968; Nicoll, 1969; Price & Powell, 1970; Mori & Takagi, 1978*a*; Jackowski, Parnevalas & Lieberman, 1978), and also between mitral cells and periglomerular cell interneurones in the case of orthodromic activation (Pinching & Powell, 1971; Shepherd, 1971; White, 1972, 1973; Getchell & Shepherd, 1975*a*, *b*; Kauer & Shepherd, 1977). These interactions are of additional general interest in view of the increasing evidence for dendrodendritic synapses in other parts of the nervous system (cf. Rakic, 1976; Pearson, 1976; Schmitt & Worden, 1979).

In order to obtain further evidence for the physiological properties of this type of synaptic interaction we have developed an isolated preparation of the turtle olfactory bulb. The viability of this preparation has been demonstrated (Nowycky, Waldow & Shepherd, 1978; Mori & Shepherd, 1979*a*; Waldow, Nowycky & Shepherd, 1981), and the basic properties of the generation of antidromic and orthodromic impulses have been described in the preceding paper (Mori, Nowycky & Shepherd, 1981*a*). We report here an analysis of the excitatory and inhibitory synaptic potentials elicited in the mitral cell of the isolated olfactory bulb by single volleys in the antidromic and orthodromic pathways. Some of the results have been briefly reported previously (Nowycky *et al.* 1978; Mori & Shepherd, 1979*a*, *b*).

METHODS

The experiments have been carried out on the olfactory bulb of the turtle, *Pseudemys scripta*. The preparation of the *in vitro* olfactory bulb, and methods for stimulating and recording, have been fully described in the preceding paper (Mori *et al.* 1981*a*).

RESULTS

Responses to lateral olfactory tract volleys

A total of seventy-three cells has been analysed for responses to volleys in the lateral olfactory tract (LOT). A typical response is shown in Fig. 1*A*. Following the spike, the membrane hyperpolarized, and there was a series of potential changes. The brief spike after-hyperpolarization (asterisk) was followed by a further hyperpolarization, a slight repolarization (double asterisk) and a final slow hyperpolarization. The two hyperpolarizing potentials are labelled I_1 and I_2 in the figure; we will show in the following analysis that they have the properties of inhibitory post-synaptic potentials (i.p.s.p.s). The I_2 component grades into a later prolonged potential, which is the subject of the following paper (Mori *et al.* 1981*b*).

The two hyperpolarizing components were independent of the spike and its after-potentials. This is shown in Fig. 1*B*, in which the hyperpolarizing components were present when the LOT shock was below threshold for eliciting an impulse in the

axon of this cell. The time relations of the two components in a population of thirty-seven cells, compared with each other and with the latency of the antidromic spike, are shown in Fig. 1 *C* and *D*. Note the close grouping of values for the onset latencies of the first synaptic component (I_1), and the delay of several milliseconds

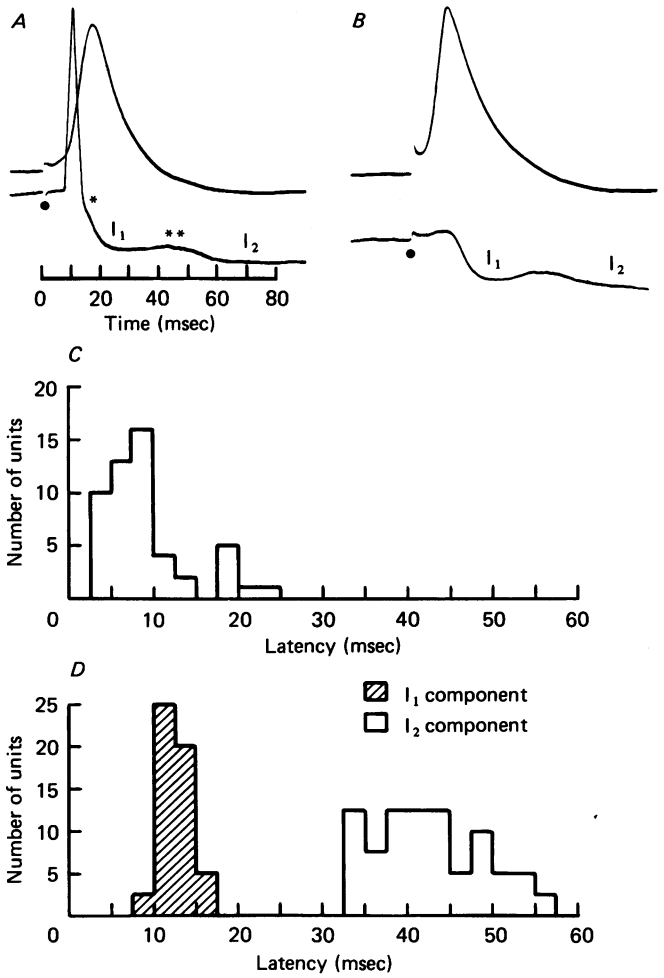

Fig. 1. Intracellular responses of mitral cells to LOT volley. *A* (lower trace), intracellular response: shock artefact (●); spike after-potential (*); first component of i.p.s.p. (I_1); repolarizing potential (**); second component of i.p.s.p. (I_2). *A* (upper trace), simultaneously recorded field potential (positivity of recording tip is upward). *B*, responses of mitral cell to LOT shock just subthreshold for axon of this cell. Time calibration same as for *A*. *C*, latencies of different antidromic spikes ($n = 50$), *D*, latencies of onset of I_1 and I_2 components of i.p.s.p.s ($n = 37$).

after the antidromic spikes. The latencies of I_2 onset were 30–50 msec following the antidromic spike, and 20–45 msec following the onset of I_1. The I_1 and I_2 components were present in all the cells of our series, either as hyperpolarizing or depolarizing (see below) responses; if not present in control recordings, they could be revealed by

current injection (see also below). The separation of the two components varied in different cells, from being quite clear (as in Fig. 1) to relatively continuous.

The properties of the hyperpolarizing potentials in relation to graded stimulation are illustrated in Fig. 2. In *A* are shown the responses to LOT volleys at two intensities, both below threshold for impulse generation in this cell. The hyperpolarizing responses began at the arrows. Their latencies decreased with the stronger

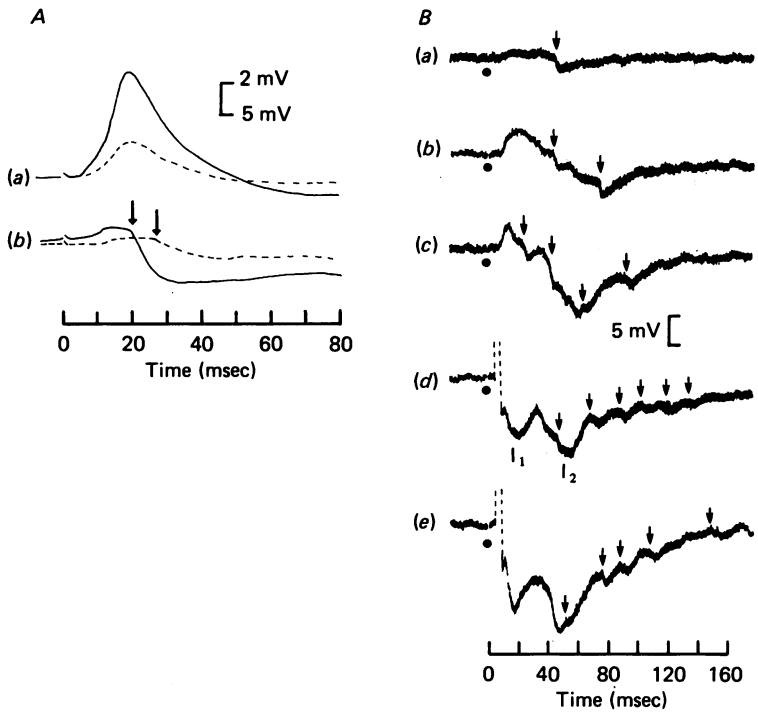

Fig. 2. Intracellular responses to graded LOT volleys. *A*, responses of mitral cell to LOT volleys at two intensities, both below threshold for axon of this cell. Onset of hyperpolarizing responses indicated by arrows. Extracellular field potentials (*a*), intracellular recordings (*b*). Tracings from original recordings. *B*, response of a mitral cell to LOT volleys, of increasing intensity from *a* to *e*. Miniature hyperpolarizing potentials indicated by arrows (see text). a.c. recordings.

volleys, and the amplitudes increased, in a graded manner. At these weak stimulus strengths the two components, I_1 and I_2, were not distinct in this cell. In twenty-eight cells the average amplitudes of I_1 and I_2 with maximal LOT volleys were 16 mV and 23 mV respectively.

The graded nature of the responses suggests that the i.p.s.p.s are built up of many small synaptic inputs. Evidence of these inputs has been obtained with high-gain a.c. recordings. The responses to weak volleys (Fig. 2*B*, *a*) were characterized by small potentials with rapid rising phases and slow decays (arrow). Slightly stronger volleys (*b*) elicited an initial depolarization (the extracellular field potential) followed by a slow hyperpolarization with superimposed small potentials (arrows). With stronger volleys (*c–e*) the threshold for spike generation was reached (*d*), and the two

components, I_1 and I_2, became evident; their separation was enhanced in these a.c. recordings (*d*).

The numerous small potentials (arrows in *B*) had abrupt rising phases and slow decays. They were a common finding in high-gain recordings from turtle mitral cells.

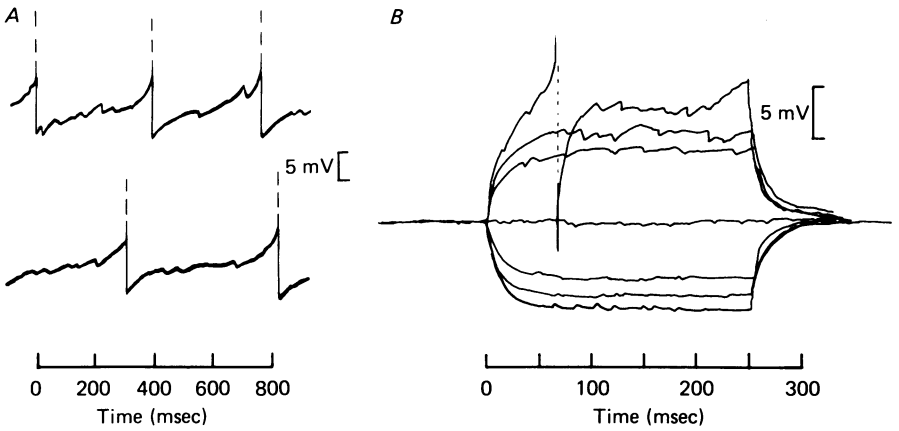

Fig. 3. Recordings of small potentials. *A*, intracellular recording of spontaneous activity; note small potentials between large spikes. *B*, different cell; effects of intracellular current injection on small potentials (see text). Traces retouched.

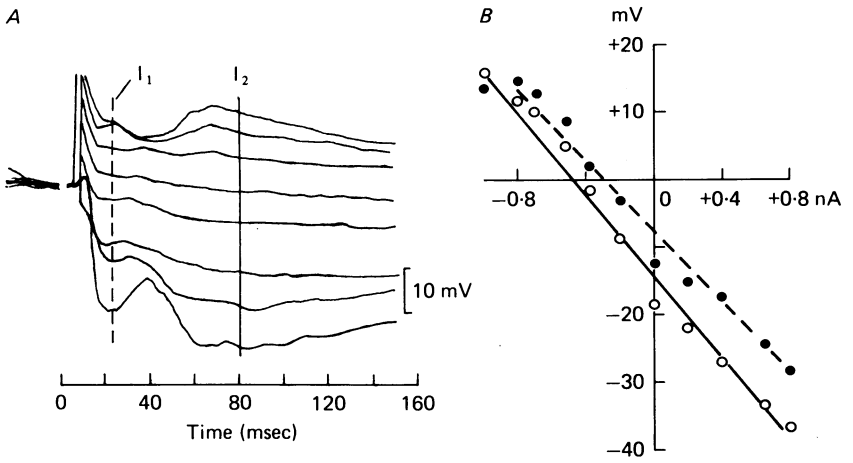

Fig. 4. Current–voltage relations of LOT-evoked response to injected currents. *A*, superimposed tracings of responses during depolarizing and hyperpolarizing currents. *B*, plots of injected currents against amplitudes of I_1 (filled circles) and I_2 (open circles) components, at times indicated in *A*.

In the evoked responses they occurred during I_1 and I_2. However, they also occurred spontaneously. Fig. 3*A* shows two records of spontaneous activity in which numerous small potentials are seen between the impulses. The effects of current injection are illustrated in Fig. 3*B*. Depolarizing current caused an enhancement of the amplitude

of the small potentials, whereas hyperpolarizing current caused a reversal in polarity of the small potentials from hyperpolarization to depolarization (bottom trace). These properties suggest that the small potentials represent miniature i.p.s.p.s (see Discussion).

The reversal potentials for the evoked i.p.s.p.s were analysed as illustrated in Fig. 4. In *A*, it can be seen that the I_2 component is clearly identifiable at all levels of injected current. Both the reversed i.p.s.p.s, and the large i.p.s.p.s revealed by membrane depolarization, had a faster rise to peak than the control response. There was also a slight asymmetry between the responses at these two extremes. The I_1 component became progressively more pronounced with increasing depolarization. The vertical lines indicate the apparent peaks of I_1 and I_2, and the amplitudes are plotted in Fig. 4*B*. It can be seen that both I_1 and I_2 have approximately linear current–voltage relations; I_1 reversed its polarity at a slightly lower current strength than I_2. The general properties of this cell were typical for experiments in eight other cells.

Responses to olfactory nerve volleys

Mitral cells typically responded to a single olfactory nerve (ON) volley with one or two impulses. Of a total of fifty-one cells, thirty-five responded with only a single spike at all intensities (Fig. 5*A*), eight always responded with two spikes in response to very strong volleys (*B*), and eight occasionally responded with a second spike to strong volleys.

A single impulse response is illustrated in Fig. 5*A*. The spike always arose from a small slow depolarization which will be referred to as E_1 (Fig. 5*C*). There was a brief hyperpolarizing after-potential, followed by a slow hyperpolarization. Two components (I_1 and I_2) in this slow wave can be seen, similar to those elicited by a LOT volley. The I_1 component in our cell population had an onset latency 7–16 msec following the onset of the depolarization giving rise to the impulse response.

A cell responding with two impulses is illustrated in Fig. 5*B*. Following the first spike and the I_1 hyperpolarization there was a rapid depolarization, which will be referred to as E_2. This led to a second spike, which occurred at approximately the time of transition from I_1 to I_2 (see also asterisk in *A*).

The synaptic properties underlying the depolarizing and hyperpolarizing responses have been analysed using carefully graded intensities of ON volleys. As shown in Fig. 5*C*, weak volleys (*a*) elicited a monophasic depolarization with relatively rapid rise and slow decay. The amplitude was graded with volley strength (*b*, *c*). Stronger volleys (*d*, *e*) elicited a single impulse which, with increasing volley strength, arose at shorter latencies. This was typical of the stimulus–response relations in our population of cells. These properties suggest that the slow depolarizing wave (E_1) is due to an excitatory post-synaptic potential (e.p.s.p.) (Mori & Shepherd, 1979*b*). Following the spike after-potential (*d*, *e*) there was a depolarizing peak (E_2; see above) before the I_2 component began. The peak of E_2 occurred at a latency of 29 msec following the onset of E_1; in a population of thirty cells the mean delay was 34 msec. In the field potential recordings (upper traces) an inflexion occurs that is closely correlated in time with the occurrence of the E_2 peak. Further analysis of these components of the e.p.s.p. will be reported elsewhere (Nowycky, Mori & Shepherd, 1981*b*).

Despite the presence of E_1 and E_2 components in the responses, I_1 and I_2 components were usually clearly present, especially at intermediate and higher ON volley strengths (cf. Fig. 5*C*). Analysis of high-gain a.c. recordings revealed small hyperpolarizing potentials during the I_1 and I_2 components. These were closely similar to those elicited by LOT volleys (Fig. 2 above).

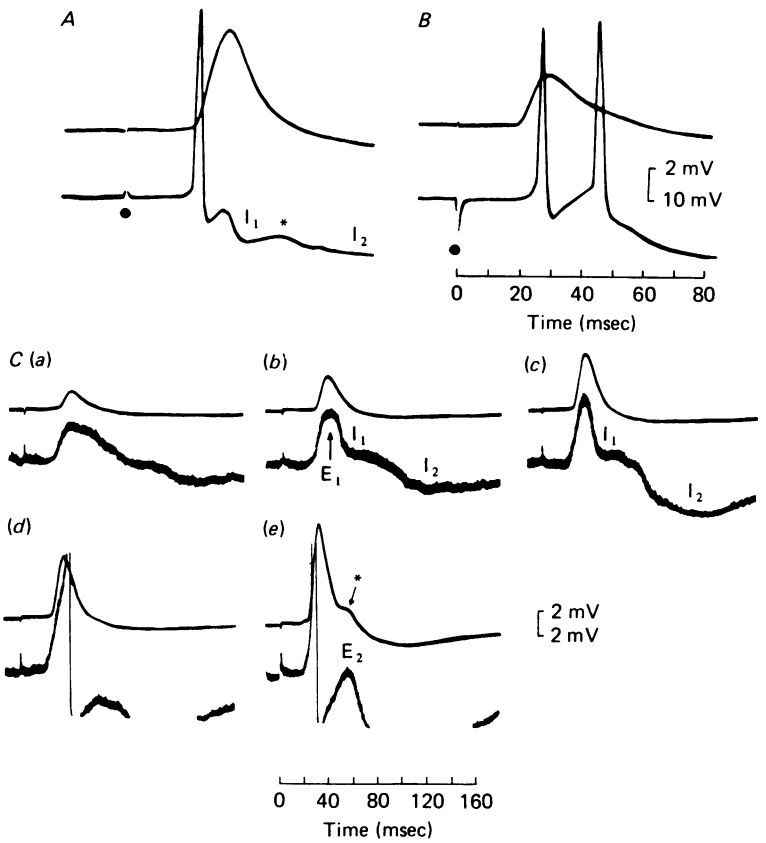

Fig. 5. Intracellular responses of mitral cells to ON volley. *A*, cell responding with single impulse to single volley. Subsequent response components I_1, repolarising wave (*) and I_2 are indicated. *B*, different cell, responding with two impulses to single volley. Calibration: 2 mV for upper trace (extracellular field potentials), 10 mV for lower traces (intracellular recordings). Spikes retouched. *C*, responses of mitral cell to graded ON stimulation. Increasing shock intensities from *a* to *e*. Upper traces: extracellular field potentials; lower traces, intracellular recordings (a.c. coupling). Spikes retouched. See text.

Experiments have been carried out with current injection to examine the properties of the response components. Typical recordings are shown in Fig. 6 of the responses of a cell to hyperpolarizing and depolarizing currents. With hyperpolarizing currents the amplitude of the E_2 component was enhanced, whereas with depolarizing currents the I_1 and I_2 components were enhanced. The amplitudes of I_1 and I_2 at the times shown by the vertical lines are plotted in *B*. It can be seen that the current–voltage

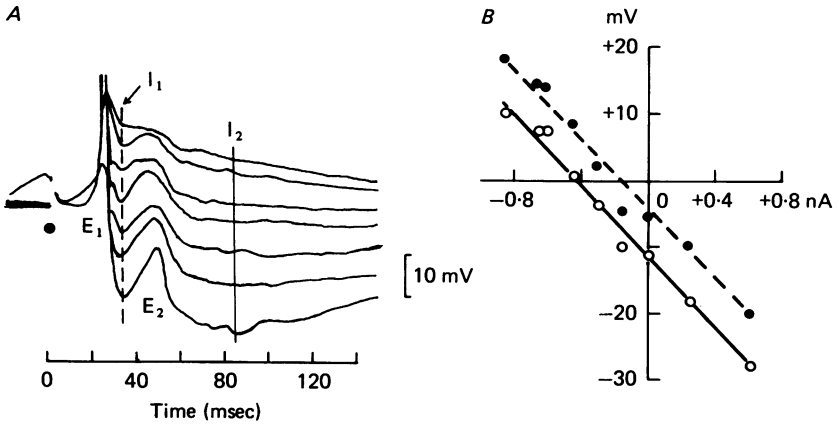

Fig. 6. Current–voltage relations of ON-evoked responses to injected currents. *A*, superimposed tracings of responses. *B*, plots of injected currents against amplitudes of I_1 (●) and I_2 (○) components, at times indicated in *A*.

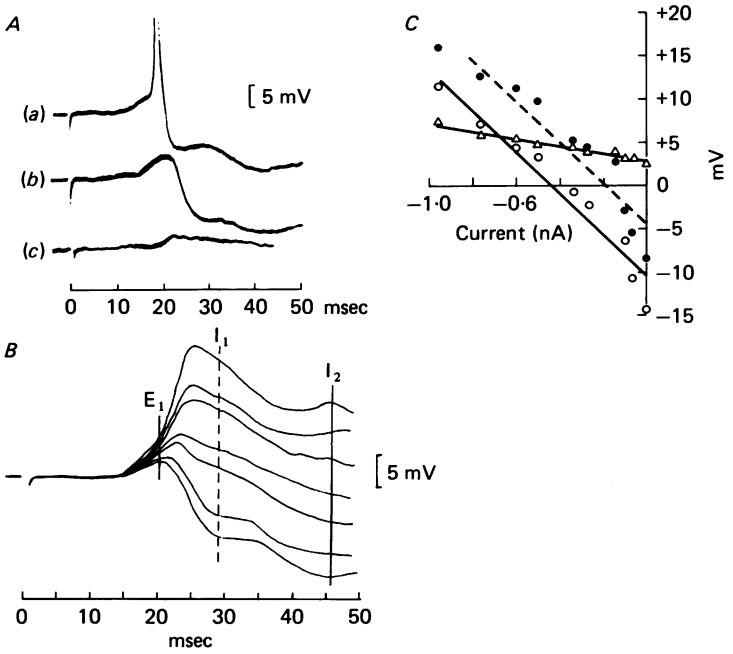

Fig. 7. Comparisons of current sensitivities of e.p.s.p.s and i.p.s.p.s. *A*, response of mitral cell to single ON volleys; highest volley strength in (*a*), lowest in (*b*), extracellular recordings in (*c*). *B*, superimposed tracings of responses of this cell during hyperpolarizing current injections of different intensities. Control response (bottom trace) is similar to (*b*) in *A*. *C*, plots of injected currents against amplitude of E_1 (△), I_1 (●) and I_2 (○) components at times indicated in *B*.

relation is roughly linear. The I_1 and I_2 components have similar slopes; the I_1 component reversed at slightly lower current strengths. These results are typical of those obtained in fifteen experiments. We conclude that the current sensitivities for the I_1 and I_2 components are closely similar to those for the corresponding components of the LOT-elicited response (cf. Fig. 4).

The current sensitivities of the i.p.s.p.s and e.p.s.p.s were compared in several experiments. This required recordings of responses on fast sweeps, and the use of

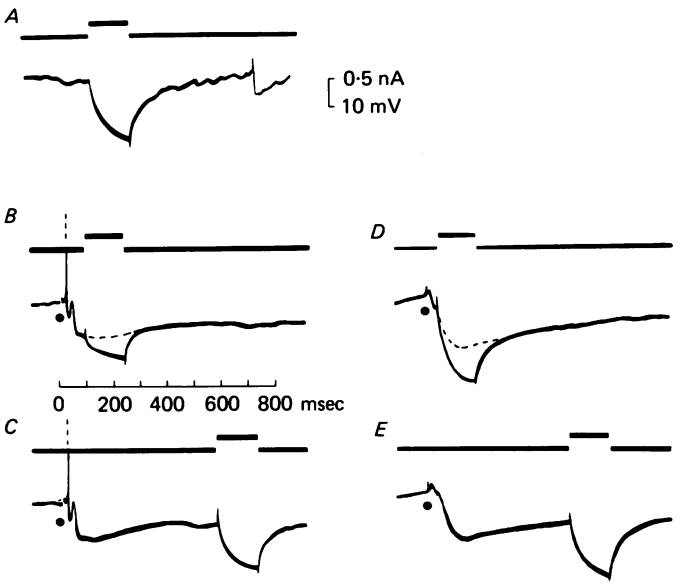

Fig. 8. Membrane conductance increases during mitral cell responses. *A*, control response (lower trace) to injected current pulse (upper trace). *B* and *C*, mitral cell responses to ON volley. Pulse injected during I_2 component (*B*) and later (*C*). *D* and *E*, responses of same cell to LOT volleys. Calibration: 0·5 nA in upper traces, 10 mV for lower traces.

subthreshold ON volleys to reveal the e.p.s.p.s. Representative responses of a mitral cell are shown in Fig. 7A. In *B* are shown tracings of seven responses obtained with different intensities of hyperpolarizing current injection, beginning with the control response (bottom trace). The amplitudes of E_1, I_1 and I_2 are plotted in Fig. 7*C*. The current–voltage relations for I_1 and I_2 resemble those already described (Fig. 6). The relation for E_1 is linear, but the slope is small, i.e. this component is relatively insensitive compared to I_1 and I_2, to the injected current. Because of this it was not possible to examine the reversal potential of E_1. The E_2 component could not be assessed adequately for its current sensitivity because of overlap with the I components.

Conductance measurements

Experiments were carried out to test the membrane conductance during the i.p.s.p.s, using brief current pulses. As described previously (Mori *et al.* 1981*a*) the responses to a current pulse consist of a slowly rising transient with a slow time

course; this was too slow to permit precise testing of conductance during the e.p.s.p. and I_1 component (but see Nowycky *et al.* 1981*b*). Testing of the I_2 component is illustrated in Fig. 8. The results show that the I_2 component of the i.p.s.p. is associated with an increase in membrane conductance. This was a general finding in all our cells. Further quantitative data on the time course of this conductance change, and its relation to the subsequent slower potential changes in the mitral cell, will be described in the following paper (Mori *et al.* 1981*b*).

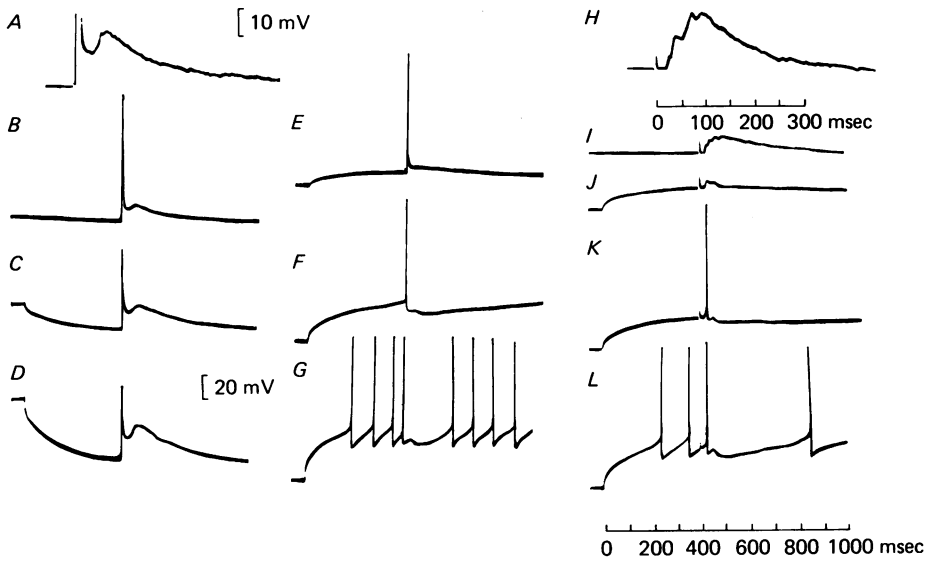

Fig. 9. Examples of depolarizing i.p.s.p.s. *A*, response to LOT volley, recorded with micropipette filled with potassium acetate. *B*, as for *A* but with slower sweep. *C* and *D*, responses during hyperpolarizing current injection. *E–G*, responses during depolarizing current injection. *H*, response of this cell to ON volley, *I*, as for *H* but with slower sweep. *J–L*, responses during depolarizing current injection. Time calibration in *H* applies also to *A*; time calibration in *L* applies to all other traces. Voltage calibration in *A* applies also to *H*; voltage calibration in *D* applies to all other traces. Spikes retouched.

Depolarizing i.p.s.p.s

In most mitral cells the i.p.s.p.s elicited by ON or LOT volleys consisted of hyperpolarizations. In 22 % of the cells, however, the corresponding potentials were depolarizing. An example is shown in Fig. 9. In *A*, the antidromic impulse elicited by a LOT volley was followed by a depolarizing wave. The responses during hyperpolarizing and depolarizing current injection are shown in *B–D* and *E–G*, respectively. Note the increase in amplitude of the depolarizing wave with membrane hyperpolarization (*D*), and decrease and reversal to the i.p.s.p. of more usual appearance with depolarizing current (*F* and *G*). The inhibitory nature of the potential is confirmed by the interruption of impulse discharge in *G*. Similar results are shown in *H–L* for the responses to ON volleys.

All of the cells showing depolarizing i.p.s.p.s had relatively high resting membrane potentials, ranging from 75 to 85 mV. These cells showed no spontaneous activity.

In some cases, following penetration by the electrode a cell would change from having a low membrane potential, with hyperpolarizing i.p.s.p., to having a high membrane potential, with depolarizing i.p.s.p. With prolonged penetration some cells gradually showed changes in the reverse direction.

The results described here were obtained with micropipettes filled with potassium acetate or potassium citrate. Reversed i.p.s.p.s were also recorded with micropipettes filled with potassium chloride; these results are described elsewhere (Nowycky *et al.* 1981*a*, *b*).

DISCUSSION

Identification of components of mitral cell responses. The results of this study show that the complex potentials that are elicited by either an ON or a LOT volley can be divided into a series of components that is highly reproducible. The components are of either an excitatory (E) or inhibitory (I) nature. The two I components, I_1 and I_2, are seen after both LOT and ON volleys, whereas the two E components, E_1 and E_2, are seen only after ON volleys. Despite the presence of the E components in the ON responses, the I components have similar time relations in both ON and LOT responses. This was shown by the results illustrated in Figs. 4 and 6; the relations between the components are summarized by the superimposed traces in Fig. 10 (*inset*). When the antidromic impulse is aligned with the orthodromic impulse, as in this Figure, the timings of the subsequent I_1 and I_2 components can be seen to be nearly identical.

Synaptic properties of hyperpolarizing responses. The postulate that the I_1 and I_2 components represent inhibitory synaptic actions on the mitral cell is supported by a variety of evidence in the present study. Both response components appeared to be composed of miniature inhibitory potentials. The components increase in amplitude with increasing stimulus strength in a graded manner. The current sensitivities and reversal potentials are similar for I_1 and I_2, and are also in the same range for the miniature potentials. When I_1 and I_2 are depolarizing, they can be reversed with depolarizing current injection. An increase in membrane conductance is clearly associated with the I_2 component, and is probably also present during the I_1 component (see Nowycky *et al.* 1981*a*, *b*).

The results are consistent with the usual criteria for the properties associated with i.p.s.p.s (cf. Eccles, 1964; Hubbard, Llinás & Quastel, 1969). The reversal potentials for the I_1 and I_2 components suggest that these components are mediated by increased conductance for ions that have their equilibrium potentials somewhat more polarized than the resting membrane potentials. Evidence implicating chloride ions in this conductance change will be presented elsewhere (Nowycky *et al.* 1981a, b). Miniature i.p.s.p.s have only rarely been reported from elsewhere in the vertebrate brain (see Eccles, Llinás & Sasaki, 1966). The relatively large amplitudes of these potentials in the mitral cell may reflect several factors: the location of inhibitory synapses at and near the cell body (cf. Price & Powell, 1970; Willey, 1973); the relatively high input resistance of mitral cells in the turtle (Mori *et al.* 1981*a*); a relatively large driving force, perhaps reflecting the level of the equilibrium potential for chloride ions (cf. Nowycky *et al.* 1981*a*, *b*).

The close correlation in timing of the I_1 and I_2 components elicited by ON and LOT

volleys suggests that these components represent similar synaptic events in the two cases. The implications of this close correlation in timing of I_1 and I_2 for interpreting the pathways generating these components will be discussed further below.

Synaptic properties of excitatory responses. The response of a mitral cell to a single LOT volley consists of a single antidromic spike and the hyperpolarizing potentials. This is similar to findings in the rabbit *in vivo* (Yamamoto *et al.* 1963; Phillips *et al.*

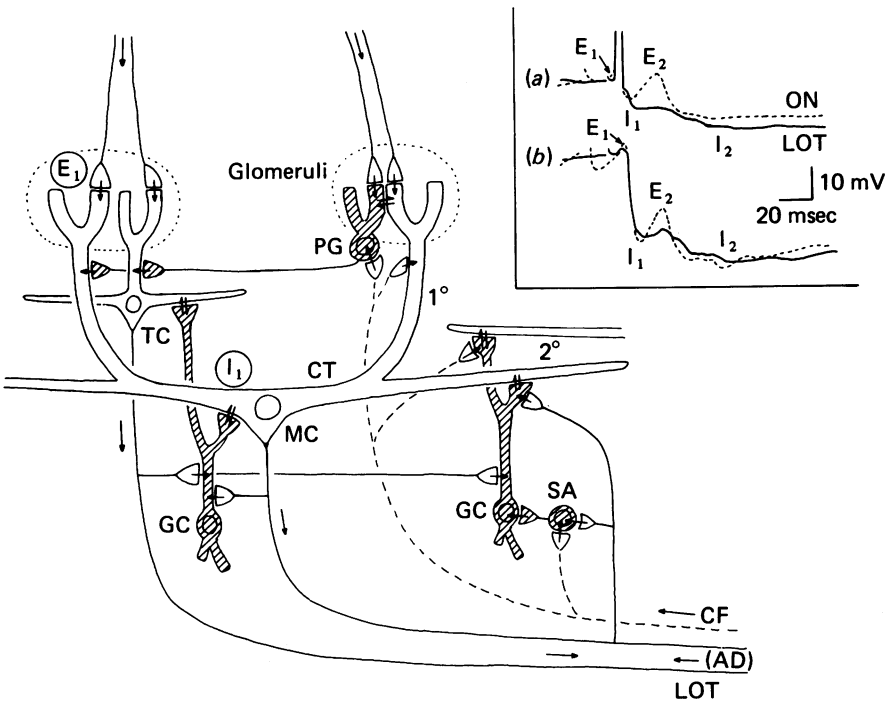

Fig. 10. Summary diagram illustrating possible sites of synaptic actions analysed in this study. AD, antidromic; CF, centrifugal fibres; CT, common trunk; GC, granule cell; LOT, lateral olfactory tract; MC, mitral cell; PG, periglomerular cell; SA, short-axon cell; TC, tufted cell, 1°, primary dendrite; 2°, secondary dendrite. Probable sites of generation of E_1 and I_1 components of synaptic potentials are indicated. *Inset*, comparison of mitral cell responses to ON and LOT volley. (*a*) Superimposed tracings of responses at normal resting membrane potential. (*b*) Responses during membrane depolarization by current injection. Same cell as figure 4 and 6. Note close alignment of corresponding components of LOT and ON responses. See text.

1963; Nicoll, 1969; Getchell & Shepherd, 1975*a*; Mori & Takagi, 1975, 1978*a*). No evidence was obtained for excitatory input to the mitral cell elicited by a shock to the LOT. This does not rule out the possibility of other input such as centrifugal fibres (cf. Mori & Takagi, 1978*b*) or excitatory recurrent collaterals (cf. Nicoll, 1971*a*), but it was not evident under the conditions of our experiments.

In contrast, the mitral cell response to an ON volley begins with a depolarization that has the properties of an e.p.s.p. These properties include a grading of rate of rise and amplitude with stimulus strength, generation of an impulse response, and

a sensitivity to current injection. The e.p.s.p. appeared to be similar, at least in its initial phase (E_1), to the e.p.s.p. in mitral cell responses in the rabbit *in vivo* (Mori & Takagi, 1975; Getchell & Shepherd, 1975*a*). The grading of e.p.s.p. amplitude presumably reflects the stimulation of increasing numbers of ON axons that converge on the olfactory glomeruli where synapses are made onto the dendritic tufts of the mitral cell.

The e.p.s.p. generated in a mitral cell by an ON volley in the turtle has a second component (E_2). This corresponds to component 3 in our original description (Mori & Shepherd, 1979*b*). It has not previously been reported in the orthodromic responses of mitral cells in other species. The E_2 component appears to be brief because it occurs between the I_1 and I_2 components. When I_1 and I_2 are blocked pharmacologically the E_2 component is found to be continuous with a prolonged depolarization that lasts through the period of E_2 and beyond. This will be described further elsewhere (Nowycky *et al.* 1981*b*).

Synaptic pathways for mitral cell responses. The pathways involved in mediating the responses of mitral cells described in this study may be summarized in relation to the schematic diagram of Fig. 10. The simplest case is that of activation of the LOT. This causes an antidromic impulse to propagate in the mitral cell axon and invade the mitral cell body. Previous studies (Phillips *et al.* 1963; Rall *et al.* 1966; Rall & Shepherd, 1968; Nicoll, 1969; Mori & Takagi, 1978*a*; Jahr & Nicoll, 1980) have suggested that the impulse in the mitral cell activates a dendrodendritic pathway for excitation of granule cells, followed by activation of inhibitory synapses from the granule cells onto the mitral cells. These reciprocal synapses are believed to provide for self and lateral inhibition of the mitral cells, as well as of tufted cells, as indicated in Fig. 10. There is evidence that the granule-to-mitral synapse is GABA-ergic (McLennan, 1971; Nicoll, 1971*b*; Ribak, Vaughn, Saito, Barber & Roberts, 1977; Halasz, Llungdahl & Hökfelt, 1979; Nowycky *et al.* 1981*a*, *b*).

We postulate therefore that the I_1 component represents the initial activation of the dendrodendritic synapses by the antidromic volley. This is consistent with the timing of onset of I_1, which is always several milliseconds later than the onset of the spike. The similarity of the properties of I_1 and I_2 suggests that to a large extent I_2 is generated by similar synaptic mechanisms, and at similar sites on the mitral cell, as I_1. In contrast to LOT activation, an orthodromic volley in the ON generates an e.p.s.p. in the glomerular tufts of a mitral cell, which then spreads to elicit the impulse response. The low current sensitivity of the E_1 component, in comparison with I_1 and I_2, suggests that the site of generation of E_1 is further from the cell body. This is consistent with the fact that the glomerular tuft and the cell body are separated by 500–800 μm (Mori *et al.* 1981*a*). All the ON input to the mitral cell is confined to the glomeruli, so that e.p.s.p. generation can be unequivocally localized to this site. The e.p.s.p. spreads electrotonically through the primary dendrite and common trunk to the cell body; it may be aided by dendritic fast-prepotentials (Mori & Takagi, 1975).

The similarity of the orthodromic I_1 and I_2 components to those evoked antidromically suggests that the dendrodendritic synapses are activated in a similar manner in the two cases. This implies that the synaptic pathways that are common in the two cases are likely to be local circuits closely related to the mitral cell, rather than longer or more distant circuits, especially circuits that would be preferentially

activated in one case to the exclusion of the other. This points to the secondary dendrites, which would be invaded in a similar manner following both an antidromic and orthodromic impulse in the cell body, as originally postulated (Rall & Shepherd, 1968). For synapses on the common dendritic trunk and primary dendrites the sequence of activation would be different in the antidromic and orthodromic directions; however, this difference would be expected to be small. The activation of dendrodendritic synapses in the glomeruli would be expected to be quite different; these synapses are located at the sites of ON input, and are therefore maximally activated by an ON volley, but to a much lesser degree, if at all, by spread of an antidromic impulse.

Other possible pathways to consider for I_2 include: an intrinsic property providing for delayed reactivation of the reciprocal synapses; pathways through mitral cell or tufted cell axon collaterals; centrifugal fibres activated by the LOT stimulus. It appears unlikely that I_2 is mediated by activation of centrifugal fibres, because these are directly stimulated by shocks to the LOT, but only very indirectly by ON volleys activating mitral cells which in turn activate olfactory cortical neurones (see Fig. 10). In most of our experiments the cortical neurones were not included in the preparation. The activation of axon collateral pathways would be different in the two cases, but the timing difference might be small, and not detectable in our experiments.

These considerations indicate that orthodromic and antidromic volleys provide for an initial activation of dendrodendritic synaptic pathways in the turtle bulb that is similar to that postulated in other species. They provide further evidence for a second phase of inhibitory activity (I_2) in these pathways that has not been previously described. It may be that the slow time course of activity in the turtle has enabled this second component to be seen more clearly; alternatively, it may represent a pathway or type of activity not present in certain of the other species. A third phase of activity, consisting of a very slow response component that follows the inhibitory synaptic potentials, is also present in the turtle, and will be described in the following paper (Mori *et al.* 1981*b*).

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