5.2 Dendrodendritic Synaptic Pathway for Inhibition in the Olfactory Bulb (1966), *Exptl. Neurol.* 14:44–56

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Anatomical and physiological evidence based on independent studies of the mammalian olfactory bulb points to synaptic interactions between dendrites. A theoretical analysis of electric potentials in the rabbit olfactory bulb led originally to the conclusion that mitral dendrites synaptically excite granule dendrites and granule dendrites then synaptically inhibit mitral dendrites. In an independent electron micrographic study of the rat olfactory bulb, synaptic contacts were found between granule and mitral dendrites. An unusual feature was the occurrence of more than one synaptic contact per single granule ending on a mitral dendrite; as inferred from the morphology of these synaptic contacts, a single granule ending was often presynaptic at one point and postsynaptic at an adjacent point with respect to the contiguous mitral dendrite. We postulate that these synaptic contacts mediate mitral-to-granule excitation and granule-to-mitral inhibition. These dendrodendritic synapses could provide a pathway for both lateral and self inhibition.

Introduction

The purpose of this paper is to bring together two recent but independent lines of evidence which suggest that there is synaptic interaction, in both directions, between the dendrites of mitral cells and the dendrites of granule cells in the mammalian olfactory bulb. By means of these synapses, mitral cell dendrites would excite granule cell dendrites, and granule cell dendrites would then inhibit mitral cell dendrites. The morphological evidence is from an electron micrographic study of the olfactory bulb (Reese and Brightman), while the physiological argument arose originally from a theoretical analysis (Rall and Shepherd) of electric potentials obtained from recent physiological studies on the olfactory bulb (15, 16).

Anatomy

Figure 1 emphasizes, for our purposes, the form and relations of the large mitral cells as seen in Golgi preparations (19). Each mitral cell has

Figures 1 and 3 were drawn by Mrs. G. Turner.

a radial, primary dendrite which receives the incoming fibers from the olfactory epithelium, and several tangential, secondary dendrites which form an external plexiform layer. Into this layer come also many branches of radial dendrites from numerous deeper lying granule cells. These

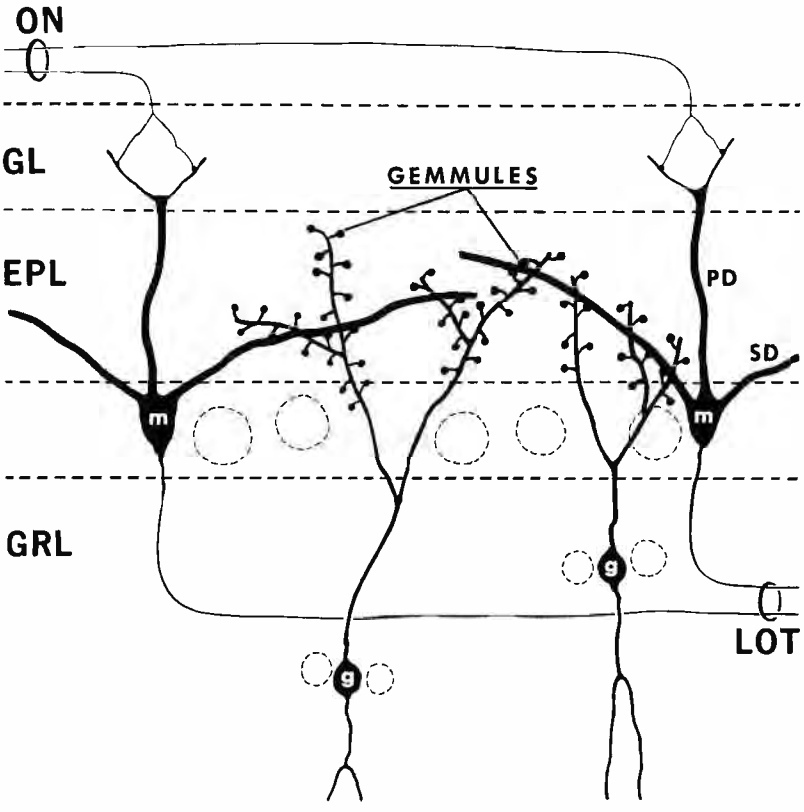

FIG. 1. Layers and connections in the olfactory bulb (adapted from Cajal, 19). GL, glomerular layer; EPL, external plexiform layer; GRL, granular layer; ON, olfactory nerve; LOT, lateral olfactory tract; g, granule cell; m, mitral cell; PD, primary mitral dendrite; SD, secondary mitral dendrite.

branches are studded with gemmules (Golgi spines) which make many contacts with the mitral secondary dendrites. Mitral dendrites resemble dendrites of other multipolar neurons in being distinctly wider than the axon, and in emerging as multiple trunks, whereas the axon is single.

Granule cell dendrites resemble certain other dendrites in bearing gemmules and being profusely branched.

Methods. Rats weighing 200-250 g were perfused through the heart with a solution of 1% OsO_4 in isotonic sodium phosphate at pH 7.0, and the olfactory bulbs were prepared for electron microscopy by conventional techniques. In order to identify cell processes, it was important to know both the region of the bulb and its orientation in each electron micrograph. This was achieved by embedding large coronal slices of olfactory bulb and sectioning them for electron microscopy only after they were examined with the light microscope. The electron microscopic results are here limited to the deeper regions of the external plexiform layer where most of the large tangential dendrites are from mitral cells. A few of these dendrites, however, are from deep lying tufted cells.

Results. Mitral and granule cell dendrites were easily identified in electron micrographs by comparing their appearance with that in previously available Golgi preparations (19). The mitral secondary dendrites were the only large processes in the external plexiform layer with a tangential and a rostral-caudal orientation (22), while the granule cell dendrites were smaller and had a radial orientation, perpendicular to the mitral dendrites. The mitral secondary dendrites were studded with numerous synaptic endings which were thought to be mostly gemmules from granule cells because their ubiquity corresponded to that of the gemmules in Golgi preparations. Also, in favorable sections (Fig. 2) and in an accurate reconstruction of one series of sections (Fig. 3) these endings were shaped like gemmules and arose from a radially oriented granule cell dendrite.

Each granule ending made one or more typical synaptic contacts with a single mitral dendrite and each synaptic contact consisted of a region of increased density and separation of the apposed cell membranes forming a cleft filled with a fibrillar dense material. Also part of each synaptic contact was a cluster of vesicles closely applied to the cytoplasmic side of either the mitral or granule cell membrane. These synaptic contacts there-

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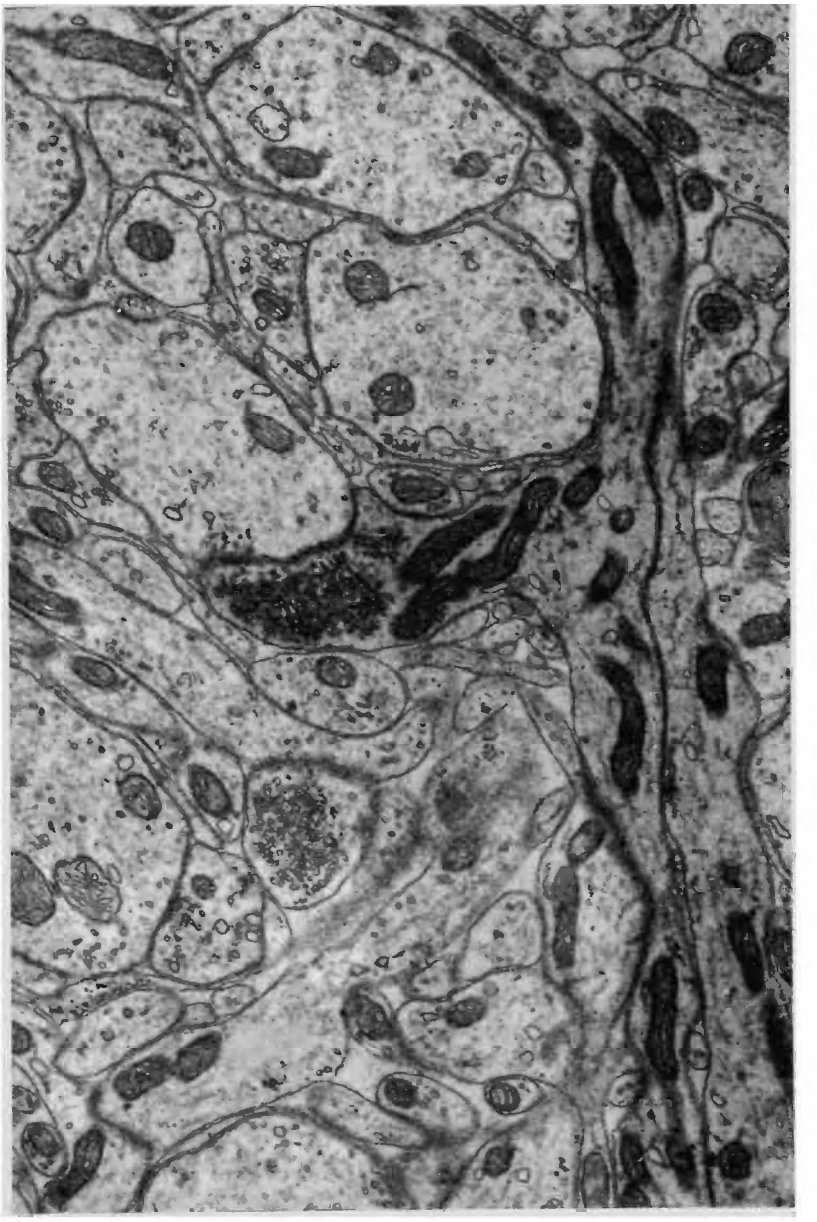
FIG. 2. Electron micrograph showing many synaptic endings on mitral secondary dendrites (large circular outlines) in the external plexiform layer of the rat olfactory bulb. One of the endings, a gemmule containing many vesicles, is in the center. A fortuitous plane of section along the axis of a granule cell dendrite (photographically darkened) demonstrates the continuity of this dendrite with the gemmule. Although this gemmule contains synaptic vesicles, no synaptic contacts are shown clearly in this plane of section. Lead citrate; × 20 000.



fore closely resembled synaptic contacts in other parts of the central nervous system (6, 14). However, the granule endings on mitral dendrites differed from typical synaptic endings in that two separate synaptic contacts with *opposite* polarities were often found side by side in the same

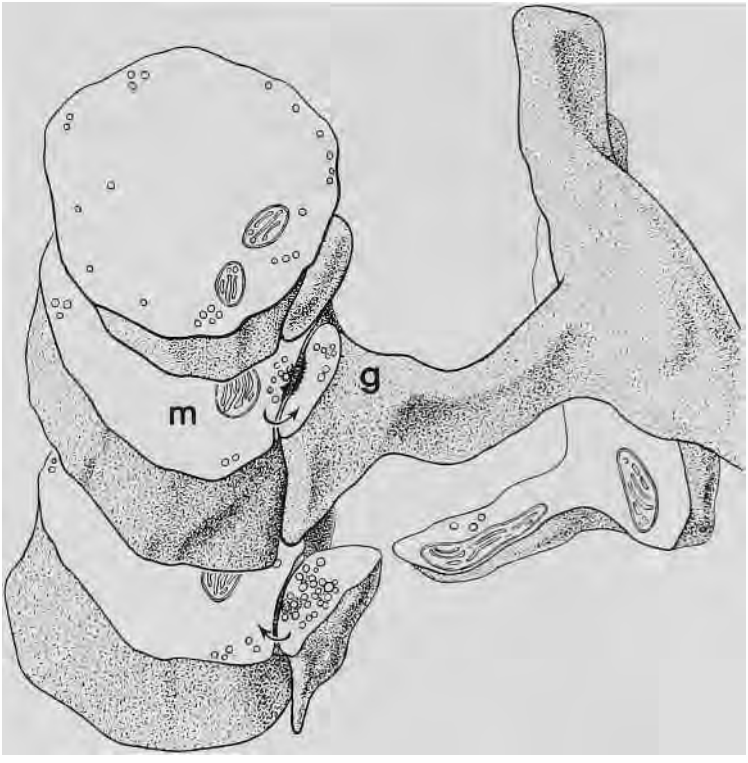


FIG. 3. Graphical reconstruction (12) of a granule synaptic ending (g) on a mitral secondary dendrite (m). The granule ending is shaped like a gemmule and arises from a granule dendrite lying approximately perpendicular to the mitral dendrite. Within a single ending are two synaptic contacts with opposite polarities (indicated by arrows). The reconstruction was made directly from a series of tracings of twenty-three consecutive electron micrographs; no sections are omitted in showing cut surfaces. Microtubules and endoplasmic reticulum are not shown. × 20 000.

ending (arrows, Fig. 3 and 4). This interpretation of polarity was made by analogy with synapses in other regions of the central nervous system where polarity depends on a grouping of vesicles at the dense segment of the presynaptic membrane (14); this interpretation implies that there is

both mitral-to-granule and granule-to-mitral synaptic transmission. That many of the granule endings appeared to make only one synaptic contact with a mitral dendrite could depend on the part of the ending sectioned (Fig. 3).

Another consistent difference in structure which correlated with the polarity of a synaptic region was a dense, filamentous material typically

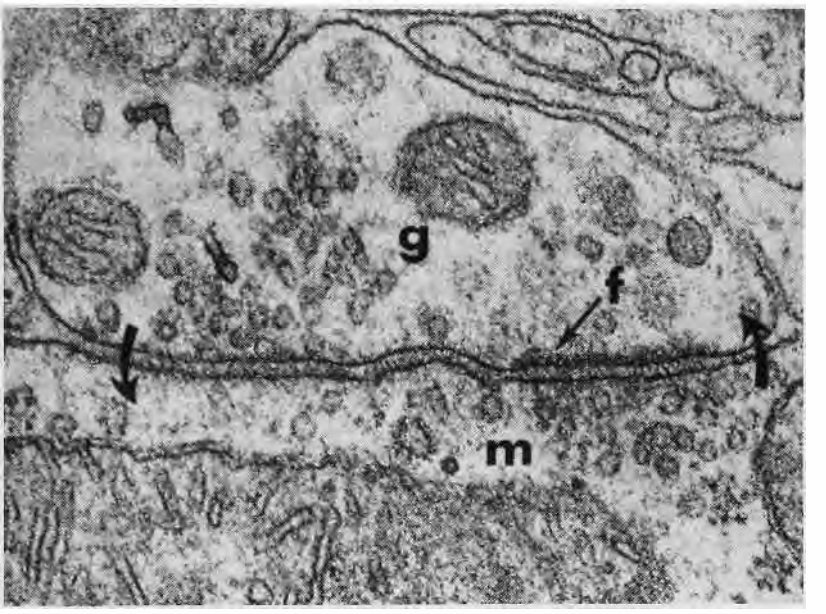


FIG. 4. A mitral secondary dendrite (m) and one of the many synaptic endings (g), presumed to be gemmules from granule cells. There are two synaptic contacts with opposite polarities (indicated by arrows). Where the polarity is from the mitral dendrite to the granule dendrite (as judged by the grouping of vesicles), a dense filamentous material (f) is attached to the postsynaptic cell membrane. Lead citrate; × 90 000.

attached to the postsynaptic cell membrane at the mitral-to-granule synaptic contacts (Fig. 4, f). In this respect, these synaptic contacts are analogous to those in axodendritic synapses in cortical areas, while the absence of a postsynaptic dense material at the granule-to-mitral synaptic contacts makes them more analogous to those in axosomatic synapses (6). Mitral-to-granule synaptic contacts also differed from granule-to-mitral contacts in having fewer vesicles near the presynaptic membrane and in having a

wider synaptic cleft. These structural differences suggest that a different function is associated with each of the two kinds of synaptic contact.

Physiology

Independent evidence for the existence and nature of connections between mitral and granule dendrites has come from electrophysiological studies of the rabbit's olfactory bulb. A weak electric shock to the lateral olfactory tract sets up an impulse volley in some axons of the mitral cells, and these mitral cells are then invaded antidromically. The mitral cells which have not been invaded may nonetheless be inhibited for 100 msec or more following the volley, and during this period, the mitral cell membrane is hyperpolarized. The variable latency of onset of the inhibition in individual mitral cells, together with its prolonged duration, suggested an interneuronal pathway between the stimulated mitral cells and their inhibited neighbors. In view of the anatomical data available at that time, it was proposed that the granule cells function as inhibitory interneurons. It was assumed that mitral axon collaterals would excite the granule cells (presumably at their deep lying dendrites and cell bodies), and that granule cell activity would then deliver synaptic inhibition to the mitral dendrites (16, 21, 26).

The new concept, that mitral secondary dendrites deliver synaptic excitation directly to granule dendrites, arose from a theoretical study in which mathematical computations were adapted specifically to a reconstruction of the electric potential distribution in the olfactory bulb following a strong shock to the mitral axons in the lateral olfactory tract. The potential pattern, as a function of both time and depth in the bulb, is very clear and reproducible (13, 16, 24) (Fig. 5).

Methods. Extensive computations were performed on a Honeywell 800 digital computer. These were based upon the mathematical neuron models presented elsewhere (17, 18). In adapting these models to the present problem, special attention was given to the concentric laminar arrangement of the mitral and granule cell populations in the olfactory bulb. Also, computations were used to assess the theoretical parameters corresponding to

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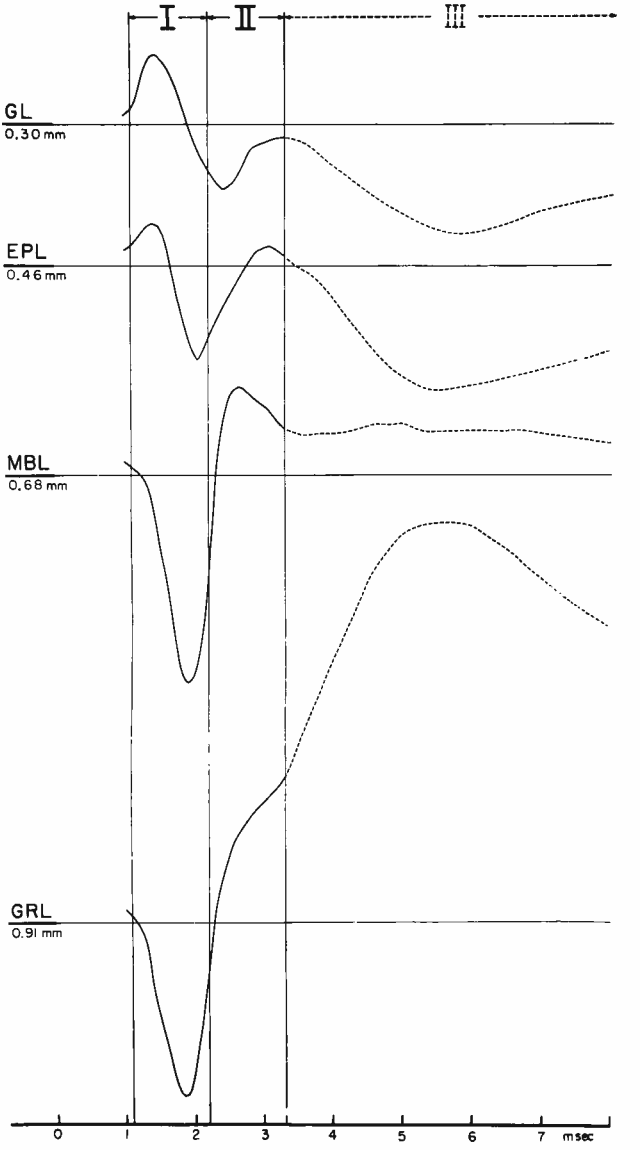
FIG. 5. Tracings of experimental recordings from rabbit's olfactory bulb following a strong shock to the mitral axons in the lateral olfactory tract (16). Periods I, II and III are indicated at top; the time from stimulus is shown at bottom. The depth of microelectrode penetration into the bulb is given at left, for each tracing; GL, glomerular layer; EPL, external plexiform layer; MBL, mitral body layer; GRL, granule



layer. Positivity of microelectrode, relative to a distant reference electrode, is upwards. Dashes distinguish period III, which we attribute primarily to granule cells, from periods I and II, which we attribute primarily to mitral cell activity.

dendritic electrotonic length, dendritic facilitation, active and passive membrane properties, axon-soma-dendritic safety factor, and the effective location of the reference electrode along a potential divider which bridges across the bulbar layers.

Results and Interpretations. To facilitate a brief description of these potentials and the interpretation derived from the reconstructions, we designate three successive time periods in Fig. 5. During period I, there is a brief negativity deep in the bulb, coupled with a positivity at its surface. This can be attributed primarily to flow of extracellular current from mitral dendrites to mitral cell bodies during the active depolarizing phase of synchronous action potentials in the mitral cell bodies. During period II, there is a brief deep positivity coupled with a brief surface negativity; this can be attributed primarily to flow of extracellular current from repolarizing mitral cell bodies to mitral dendrites which have been depolarized, either by passive electrotonic spread, or by active impulse invasion from the cell bodies.

During period III, there is a large positivity centered deep in the granule layer of the bulb, together with a large negativity centered in the external plexiform layer. This implies a substantial flow of extracellular current from the depths of the granule layer radially outward into the external plexiform layer. The population of cells which generates this electric current must possess a substantial intracellular pathway for the return flow of current from the external plexiform layer, through the mitral body layer, into the depths of the granule layer. This requirement is satisfied by the large population of granule cells, but not by the mitral cells. Furthermore, the potential distribution during period III could be reconstructed by assuming that there is a strong membrane depolarization of the granule dendrites in the external plexiform layer, coupled with essentially passive membrane in their deeper processes and cell bodies.

Because this depolarization of granule dendrites would occur in the region of contact with many mitral dendrites, and at a time just after the mitral dendrites were depolarized, it was logical to postulate that the mitral secondary dendrites provide synaptic excitatory input which depolarizes granule dendrites. Furthermore, because period III corresponds with the onset of mitral cell inhibition, the granule dendritic depolarization is both well timed and well placed to initiate synaptic inhibitory input to the mitral dendrites. Thus, the theoretical study led us to expect that dendrodendritic synaptic contacts would subsequently be found that could mediate both mitral-to-granule excitation and granule-to-mitral inhibition.

These have been discovered independently in electron micrographs of the mitral-granule endings as presented here. These findings also appear to be supported by other authors (1, 9).

Discussion

In summary, the sequence of events following impulse discharge in mitral cells is viewed as follows: Depolarization spreads from the mitral cell bodies into the mitral secondary dendrites; this membrane depolarization activates excitatory synapses which depolarize granule cell dendrites; the synaptic depolarization of granule dendritic membrane then activates granule-to-mitral inhibitory synapses. The resulting hyperpolarization and inhibitory effect in the mitral dendrites might be prolonged by sustained action of inhibitory transmitter.[2]

This schema suggests a pathway in which inhibition is mediated by nonpropagated depolarization of dendritic trees rather than by conduction, in the usual manner, through axons. Because these granule cells have no typical axons, it seems likely that they may function without generating an action potential. Computations with the theoretical model indicate that synaptic depolarization of the granule dendritic membrane can account for the observed potentials. However, we do not exclude the possibility that this depolarization could be augmented by a weak, active, local response. Neither can we completely exclude the possibility that the synaptic potential in the dendrites might cause occasional firing of impulses at the granule cell bodies. A nonpropagated depolarization of the deep-lying granule dendrites should produce a potential distribution opposite to that of period III; this situation has been previously reported and so interpreted (25). That graded amounts of granule dendritic depolarization could be responsible for graded release of inhibitory synaptic transmitter seems reasonable in view of experimental results obtained with presynaptic polarization at neuromuscular junctions (2, 11).

The granule cells can serve as inhibitory interneurons in a more general sense than in the schema proposed above, because their deeper lying processes receive input from several sources (19). Our emphasis upon the synaptic input in the external plexiform layer is not meant to exclude the importance of these other inputs in other situations. In fact, the granule

[2] Under conditions such as those in period III in Fig. 5, there is an additional inhibitory effect exerted upon the mitral cells. The extracellular current and potential gradient generated by the granule cells would have an anodal (hyperpolarizing) effect at the mitral cell bodies, coupled with a cathodal effect at the dendritic periphery.

cell is strategically situated to enable its inhibitory activity to represent an integration of several inputs.

It has generally been assumed that dendrites do not occupy presynaptic positions. However, in the olfactory bulb, both granule and mitral dendrites have pre- as well as postsynaptic relationships to each other. This feature is also found in the glomeruli of the olfactory bulb where dendrites, which are postynaptic to palisades of incoming axons, are presynaptic to other structures (20). Expanding the concept of dendrites to include pre- as well as postsynaptic functions adds new possibilities in the interpretation of sequences of synaptic contacts found elsewhere in the nervous system where an ending which is presynaptic to one process is postsynaptic to another (3, 7, 10, 23). For example, such a sequence of synaptic contacts might include a dendrodendritic rather than an axoaxonic contact. Because the morphological evidence for presynaptic inhibition has depended on the identification of axoaxonic synaptic contacts, some of this evidence may need re-examination.

It has long been recognized that the retinal amacrine cell and the olfactory granule cell are very similar with respect to external morphology (19). Recent findings in the retina indicate that arrangements of synaptic contacts there are similar to those in the olfactory bulb and involve the amacrine cells which, like the granule cells, lack an axon (5, 10). It will be interesting, therefore, to see whether the amacrine cells provide a similar dendrodendritic inhibitory pathway.

Because of the importance of adaptation and lateral inhibition in sensory systems (8), it is noteworthy that the mitral-granule dendrodendritic synapses provide an anatomical pathway for such inhibitory effects. From the Golgi preparations, it appears that the secondary dendrites of each mitral cell must contact the dendrites of many granule cells, and that each granule dendritic tree must contact the dendrites of many mitral cells. Thus, each time a mitral cell discharges an impulse, its dendrites deliver synaptic excitation to many neighboring granule dendritic trees, and these, in turn, deliver graded inhibition to that mitral cell and many neighboring mitral cells. Such lateral inhibition can contribute to sensory discrimination by decreasing the level of *noisy* activity in mitral cells neighboring the activated mitral cells; also, the self-inhibition would limit the output of the activated mitral cells. When there is a widespread, intense sensory input, the entire granule cell population can exert an adaptive kind of inhibitory effect upon the entire mitral cell population. Such features are in accord with physiological findings (4). Interaction between the

mitral and granule cell populations can also provide a basis for rhythmic phenomena; as the granule cell population begins to inhibit the mitral cell population, this begins to cut off a source of synaptic excitatory input to the granule cell population; later, as the level of granule cell activity subsides, this reduces the amount of inhibition delivered to the mitral cells, and permits the mitral cells to respond sooner to the excitatory input they receive.

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