THE MITRAL AND SHORT AXON CELLS OF
THE OLFACTORY BULB

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SUMMARY

A description is given of the mitral and short axon cells of the olfactory bulb of the rat from Golgi material examined with the light microscope and from material examined with the electron microscope. The mitral cells are large neurons with primary and secondary dendrites which both extend into the overlying external plexiform layer, although only the primary dendrite enters the glomerular formations. No predominant antero-posterior orientation of the secondary dendrites has been found. Within the glomeruli the mitral cell dendrites are in synaptic contact with the olfactory nerves and also with the periglomerular cells, but elsewhere the only synapses on the mitral cells are the 'reciprocal synapses' with the granule cells. Synaptic-type vesicles are found in all parts of the mitral cells, including the axon initial segments; they appear to be especially concentrated in the distal portions of the dendrites.

Several types of short axon cells have been found in the granule cell layer in Golgi-impregnated material. Their cell bodies can also be distinguished with the electron microscope, and from previous work it is probable that the axons of at least some of these cells form flattened-vesicle symmetrical synapses upon the granule cells.

INTRODUCTION

In classical descriptions of the olfactory bulb 3 distinct types of cells have been recognized in the deeper layers: the mitral, granule, and short axon cells (Cajal, 1911). Of these neurons, the granule cell has long been known to be morphologically unusual in not having a typical axon, and recent electron-microscopic studies have shown that it participates in unusual reciprocal synapses with the mitral cells (Hirata, 1964; Andres, 1965; Rall, Shepherd, Reese & Brightman, 1966). A description of this cell and of the synapses related to it has been given in previous papers (Price & Powell, 1970a–e). Although the main features of the mitral cell, which is the major efferent neuron of the bulb, are well known, both from light microscopy (Cajal, 1911) and electron microscopy (Andres, 1965), a more complete description is required before it is possible to make a synthesis of the synaptic organization of the deeper layers of the bulb, and particularly of the interrelationships between individual mitral cells. In addition, certain tentative suggestions which have recently been made in regard to the orientation of the dendrites of the mitral cells (Shepherd, 1966) deserve further consideration, as they would, if established, have important implications concerning the mechanism of integration in the olfactory pathway.

The third cell type, the short-axon cell, has been largely ignored, both in recent descriptions of the morphology of the bulb (Valverde, 1965; Andres, 1965), and in the interpretation of functional studies. There is, however, evidence to suggest that it
might be a second interneuron related to specific afferent fibres (Yamamoto, Yamamoto & Iwama, 1963).

For these reasons certain observations on the mitral and short axon cells will be presented in this paper. They have been made on extensive material collected in the course of other studies on the olfactory bulb (Price & Powell, 1970a–c).

MATERIAL AND METHODS

For electron microscopy, young rats (not more than 2 months old) were perfused with a mixture of 1% glutaraldehyde and 4% paraformaldehyde; after the brain was removed from the skull, the olfactory bulbs were cut into small blocks, post-fixed in osmium tetroxide, dehydrated and embedded in Araldite. Sections 1 μm thick were cut for orientation purposes and stained with methylene blue and azure II (Richardson, Jarett & Finke, 1960). Ultrathin sections were then cut on a Porter-Blum MT-2 ultramicrotome, and stained with lead citrate and uranyl acetate.

For light microscopy, material was prepared by the Golgi-Cox and the Golgi-Kopsch methods.

OBSERVATIONS

Both the mitral and short axon cells have been studied with the electron microscope and with the light microscope. For each cell type, therefore, a description of the cells as seen with the light microscope will first be given, followed by a description based on electron-microscopic observations.

The mitral cells

These are the largest cells in the olfactory bulb; the cell bodies have a transverse diameter of about 20 μm and a vertical diameter of 30 μm, although these values vary considerably from one cell to another. From the cell somata, which lie in the relatively

Fig. 1. Camera lucida drawings of mitral cells. Note the varicose swellings on the distal portions of the secondary dendrites.
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compact mitral cell layer, the smooth primary and secondary dendrites pass superficially into the external plexiform layer (Fig. 1). The primary dendrites, which are somewhat larger than the secondary dendrites, extend more or less radially across the plexiform layer, and enter the glomerular formations in the superficial layer of the olfactory bulb. In the rat, the distance from the origin of these dendrites on the cell body to the point at which they enter the glomeruli is of the order of 200–300 \( \mu m \).

The secondary dendrites pass obliquely through the external plexiform layer, becoming more closely parallel to the layer of mitral cells as they extend farther from their cell bodies. These dendrites branch occasionally, and can be seen in Golgi sections to become thinner, and often varicose near their terminal end. They are at least as long as the primary dendrites; although it has not been possible to measure the length of a complete dendrite with confidence, portions of the secondary dendrites have been found to be up to 300 \( \mu m \) long.

In both Golgi and Nissl material no obvious difference has been found between frontal and sagittal sections in the number of secondary dendrites seen, or in their orientation. This has been especially notable in the 1-\( \mu m \), ‘thick’ sections of Araldite-embedded material, stained with methylene blue and azure II, which have been used for orientation of electron-microscopic sections; in these sections all parts of the neuron can be clearly seen, and mitral cell dendrites, whether cut transversely or longitudinally, can be clearly recognized. In both of these planes of section, the majority of those dendrites appear to be cut obliquely or transversely, but there is always a substantial fraction which have been cut longitudinally, as if the secondary dendrites were radiating from the cell body more or less equally in all directions.

The axons of the mitral cells invariably arise from the deep side of the cell, and pass into the granule cell layer deep to the mitral cells. Although axons of any length attached to the cell body are difficult to trace in the Golgi material which has been used, the axonal initial segment is often seen, both in this material and in the sections of Araldite-embedded material. In the latter, the axon hillock stands out as a relatively clear region with little or no Nissl material, and this lightness continues into the initial segment. The initial segment extends for 15–20 \( \mu m \) from the perikaryon before it suddenly narrows and becomes myelinated. The mitral cell axons collect into bundles of myelinated fibres which pass caudally through the granule cell layer toward the olfactory peduncle.

Using the electron microscope, the perikarya of the mitral cells can easily be recognized by their position immediately deep to the external plexiform layer, and by their large size and characteristic appearance (Andres, 1965). They are similar to other large neurons, with a large, pale nucleus and prominent nucleolus, surrounded by relatively abundant cytoplasmic (Fig. 3). Two additional features which distinguish these cells from the other cells in the deeper layers of the olfactory bulb are the lack of any marked cytoplasmic indentation into the nucleus (although the outline of the nucleus is usually slightly uneven) and the well ordered arrangement of the rough endoplasmic reticulum into multilaminar Nissl bodies.

The dendrites of the mitral cells are commonly seen extending from the superficial side of the perikarya into the external plexiform layer. In this layer they can be
recognized again by their relatively large size, and by the regular arrangement of their neurotubules (Fig. 7). The primary and secondary dendrites can usually be distinguished by their orientation, as the primary dendrites pass radially from their cell bodies to the glomerular layer, while the secondary dendrites run more or less parallel to the mitral cell layer, and therefore cross the primary dendrites at right angles. In any one section, most of the secondary dendrites are cut transversely, with a smaller number cut obliquely and longitudinally. No consistent or marked difference has been observed between frontal and sagittal sections in the number of secondary dendrites which are cut transversely or longitudinally. The outline of the primary and most of the secondary dendrites is relatively regular, but occasionally a dendrite is found which has an irregular, varicose outline (Fig. 8); as the secondary dendrites have often been observed in Golgi material to be varicose near their end, the varicose dendrite segments seen with the electron microscope have been identified as being the more distal portions of the secondary dendrites. Both the primary and secondary dendrites are often covered by thin glial lamellae over portions of their length but this covering is very incomplete; not only is it interrupted for synaptic contacts, but it is absent in many places, where the dendrites come into direct apposition with the adjacent neuronal profiles. In the glomerular formations the primary dendrites branch repeatedly; the smaller terminal dendrites in this region closely resemble the irregular end portions of the secondary dendrites.

Apart from the regular array of neurotubules, there are several inclusions in the dendrites, including multivesicular bodies, ribosomes and, more surprisingly, grape-like clusters of agranular endoplasmic reticulum, and synaptic vesicles. Andres (1965) noted that the synaptic vesicles are often in very close relation to the agranular endoplasmic reticulum and he suggested that some, at least, of the vesicles might bud off this endoplasmic reticulum. The synaptic vesicles are found in all parts of the dendrites, but they increase in number markedly in the smaller terminal portions of the primary and secondary dendrites. These vesicles are predominantly spheroidal, and there is evidence which suggests that they may be slightly larger than the spheroidal vesicles found in many of the axon terminals in the olfactory bulb (Price & Powell, 1970).

The synaptic vesicles are associated with the unusual 'reciprocal' synapses between the mitral cell dendrites and the spine-like gemmules on the peripheral processes of the granule cells of the olfactory bulb (Hirata, 1964; Andres, 1965; Rall et al. 1966; Price & Powell, 1970b). These synapses, which are oriented both from dendrite to gemmule and from gemmule to dendrite, are found on all of the dendrites of the mitral cells, whether primary or secondary (Figs. 7, 8). Thus, in all cases in which a reasonable length of a primary dendrite can be identified by its size and orientation, one or more reciprocal synapses are found upon it, and, conversely, no relatively long portion of any mitral cell dendrite has been found which did not have a reciprocal synapse upon it. There does, however, appear to be a greater concentration of these synapses on to the smaller dendrites, so it is possible that there is a greater synaptic influence on to the secondary dendrites than on to the primary dendrites at least in the external plexiform layer.
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In the glomeruli, the mitral cell dendrites receive synapses from the terminals of the olfactory nerves which pass to the olfactory bulb from the nasal cavity. These synapses have asymmetrical synaptic thickenings and spheroidal synaptic vesicles. In addition to this, the dendrites come into synaptic relation with other neuronal processes, some of which arise from the periglomerular cells. The detailed relations of the periglomerular and other processes in the glomeruli are, however, complex and are now being investigated (A. J. Pinching, personal communication).

Reciprocal synapses are also found on the cell somata of mitral cells, and on the basal portions of the dendrites and the axon hillock. The basic structure of these synapses is the same as that of the reciprocal synapses on the dendrites, although an additional component is often found on the mitral cell side. This consists of a flattened sac with very closely opposed membranes, so that unless the section is normal to the membrane it appears as a solid bar. These sacs are located precisely opposite a portion of the asymmetrical synaptic membrane thickening, i.e. associated with that half of the synapse which is oriented from mitral to granule cell (Fig. 4). In serial sections it has been found that the sac never occupies more than approximately half of the area opposite the asymmetrical thickening, and synaptic vesicles are always found associated with the other part of the synaptic thickening, although vesicles are not found opposite that part of the thickening occluded by the sac. In serial sections, and in fortunate single sections, the flattened sacs are found to open into portions of the granular endoplasmic reticulum.

It may be noted that these flattened sacs are very similar to the subsynaptic sacs found in the spinal cord (Gray, 1962), and the cerebellar Purkinje cells (Eccles, Ito & Szentágothai, 1967). There is also a close resemblance between these sacs and the outer component of the subsurface cisternae (Rosenbluth, 1962; Siegesmund, 1968), and it is therefore of interest that subsurface cisternae are very commonly found in the perikarya and basal dendrites of the mitral cells. As elsewhere, these organelles consist of an outer flattened sac closely associated with the cell membrane, and an inner complex of one to several open sacs which have ribosomes attached to their cytoplasmic surface, and are continuous with the granular endoplasmic reticulum. In a few examples, vesicles are present between the different sacs (Fig. 5).

The axon hillock-initial segment of mitral cells has been frequently recognized, arising from the deep side of the perikarya and passing into the granule cell layer. The axon hillock has the typical pale appearance, due to a relative lack of ribosomes and granular endoplasmic reticulum, and the initial axonal segment has the characteristic bundles of neurotubules and dense undercoating of the plasma membrane (Fig. 9) which have been described in the cerebral cortex and elsewhere (Palay, Sotelo, Peters & Orkand, 1968; Peters, Proskauer & Kaiserman-Abrams, 1968; Jones & Powell, 1969a). The axon hillock varies considerably from one cell to another. In some cells the hillock is a broad cone-shaped region in the deep part of the perikaryon, from which the initial segment arises directly, but in others the hillock appears to be drawn out into a long process extending for a variable distance (up to several microns) from the perikaryon. This latter process has a very regular array of neurotubules and resembles a dendrite, except that it is considerably smaller than the proximal portions
of the mitral cell dendrites, and does not have as many inclusions, being relatively free of ribosomes and granular and agranular endoplasmic reticulum. The initial segment appears to be a continuation of this 'drawn-out axon hillock'; when followed in serial sections, the character of the process changes directly from that of the axon hillock to that of the initial segment. Reciprocal synapses are often seen on the axon hillocks, whether they are part of the perikarya or drawn out into a separate process, although these synapses do not seem to be more common here than elsewhere on the perikarya. No synapses of any sort, however, have ever been found on to the initial segment itself.

There is an additional feature apart from those described elsewhere which appears to be characteristic of the initial axonal segments of these cells, namely the presence of a relatively large number of vesicles, of several different types. The most common and striking of these are agranular vesicles which closely resemble the spheroidal synaptic vesicles in the mitral cell dendrites which are associated with the reciprocal synapses. These are scattered along the length of the axon, usually near the plasma membrane, although they have not been found in association with a synaptic thickening. In addition to these, there are usually a large number of dense-cored vesicles, and also vesicles which closely resemble the alveolate vesicles described by Palay (1963) in relation to the Golgi apparatus, or the prickly fringe vesicles ('Stachelsaumbesatz') described by Andres (1965). In agreement with the latter author (who described these vesicles in the perikarya of mitral cells), the last type of vesicles can often be seen attached to the plasma membrane, and opening into the extracellular cleft (Fig. 11).

Finally, it should be noted that, outside the glomerular formations in the superficial layers of the olfactory bulb, all of the synapses found upon the mitral cells are reciprocal synapses. Although in single sections synapses are seen which appear to be oriented only on to or away from the mitral cell dendrites or somata, when followed in serial sections these synapses have invariably been found to have a second component oriented in the opposite direction.

The short axon cells

These cells, which are intermediate in size between the granule cells and the mitral cells (their average diameter is about 15 μm), lie sparsely scattered throughout the granule cell layer of the olfactory bulb; they may be identified by their size, position and dendritic arrangements. Although they are much less common than the granule cells, the short axon cells are not rare, as one to several of them are found in most well-impregnated Golgi sections, and also in the 1-μm sections of Araldite-embedded material. In the latter, these cells may be seen to have large, pale nuclei, surrounded by a moderate amount of cytoplasm, in marked contrast to the small, dark nuclei and very thin perikarya of the granule cells. From material impregnated with the Golgi method, Cajal and his contemporaries distinguished 4 types of short axon cells on the basis of their dendritic trees and axonal ramifications (Cajal, 1911). The most distinctive of these, and the one which has been most readily identified in the present study, has numerous, very spiny dendrites, which emerge from all sides of the cell body (Fig. 2B). The cell body and the most proximal parts of the dendrites are devoid of
spines, however. Axons in continuity with the cell body have only rarely been impregnated in our material, but according to Cajal (1911) the axons of these spiny cells are of considerable length, although they remain within the granule cell layer. The other types of cells are relatively spine-free, although they usually have a few irregular spines; these spines are most common on the distal parts of the dendrites, but may occasionally be found on the proximal portions, or even on the cell body (Fig. 2A, C, D). The 2 most common types of these relatively spineless cells are very similar to each other, and Cajal's principal basis for distinguishing them is the layer

![Fig. 2. Camera lucida drawings of different types of short axon cells. See text for details.](image)

in which their axons ramify. One of these types of cells, referred to by Cajal as Golgi cells, has an axon which ramifies only in the granule cell layer, while the axons of the other type (named Cajal cells) extend through the mitral cell layer into the external plexiform layer. The Golgi cells also tend to be more stellate, with dendrites extending in all directions, while the Cajal cells are more fusiform, with dendrites arranged perpendicular to the mitral cell layer. Both stellate and perpendicular fusiform cells have been found in the present material (Fig. 2A, C). The fourth type of short axon cell described by Cajal is also fusiform, but its dendrites extend parallel to the mitral cell layer (Fig. 2D); the bodies of these cells are situated in the internal plexiform layer, immediately deep to the mitral cells. Cajal considered that the axons of these cells entered the external plexiform layer.

With the electron microscope only cell somata of the short axon cells can be identified with certainty, and it is therefore not possible to distinguish different types
of these cells. All the cells which have been identified have basically the same characteristics, and can be recognized by their appearance, which is quite distinct from that of the granule cells. The short axon cells have relatively pale nuclei, which are invariably indented by long fingers of cytoplasm; this indentation of the nucleus is one of the most characteristic features of the short axon cells. The cytoplasm surrounding the nucleus is considerably more abundant than in the granule cells, but less so than in the mitral cells, and contains a large number of ribosomes and other organelles, which give it a somewhat 'granular' appearance. The Golgi apparatus is usually well developed, and there is a considerable amount of granular endoplasmic reticulum, although the latter has never been found to be arranged into well-organized, laminated Nissl bodies. Mitochondria and lysosomal dense bodies are common. When dendrites have been cut in continuity with the cell somata, they are seen to have the same organelle-rich, 'granular' cytoplasm as the perikarya.

Synapses are occasionally found on the perikarya, and on the basal dendrites. On the perikarya such synapses as have been found have had symmetrical synaptic membrane thickenings (type II of Gray, see Colonnier, 1968) and relatively small, flattened vesicles; synapses with asymmetrical synaptic thickenings (type I of Gray) and spheroidal vesicles have also been found on basal dendrites (Fig. 14), in addition to symmetrical, flattened vesicle synapses (Fig. 13).

Although neither the dendrites nor the axons of the short axon cells have been identified away from the cell bodies, there is evidence that the axon terminals of at least some of these cells form synapses with symmetrical synaptic thickenings and flattened vesicles with the granule cells. It should be noted that the flattened vesicles in these axon terminals, as well as those in the axon terminals on to the short axon cells, are considerably smaller than the spheroidal vesicles associated with asymmetrical synapses. It has been shown that these vesicles are significantly smaller than the flattened vesicles within the processes of the granule cells, which are approximately the same size or larger than the spheroidal vesicles (Price & Powell, 1970b). Synapses of this type (small flattened vesicles) in the olfactory bulb have been considered to be intrinsic to the bulb, as they do not degenerate after very large lesions immediately behind the bulb, and because all of the extrinsic afferent fibres to the olfactory bulb have been found to form asymmetrical synapses. Although some of the asymmetrical synapses with spheroidal vesicles in the olfactory bulb are also intrinsic, these are very probably formed by the axon collaterals of the mitral cells (see Price & Powell, 1970b).

**DISCUSSION**

The principal purposes of the present paper have been to collate isolated observations on the mitral cells into a single description and to emphasize the presence of the short axon cells. Both of these are considered to be necessary for a complete description of the synaptic organization of the olfactory bulb.

The tufted cells, which have usually been considered to be closely similar to the mitral cells, have not been considered here because they may form a heterogeneous
population; while the deeper tufted cells are probably similar to the mitral cells (and morphologically, at least, they are identical), it is possible that the more superficially placed tufted cells may be different (Valverde, 1965). There is some evidence to suggest that a proportion of these cells may correspond to the short axon cells (A. J. Pinching, personal communication).

In the present material, whether examined with the light or electron microscope, no indication has been found of an antero-posterior orientation of the secondary dendrites of the mitral cells; the number of these dendrites which were cut longitudinally or transversely in fact was more or less the same whether the olfactory bulb was cut coronally or sagittally. We have therefore been unable to confirm the earlier report by Shepherd (1966) that these secondary dendrites are aligned along the antero-posterior axis of the bulb.

Although the mitral cells are in most respects similar to other large neurons, one strikingly unusual feature is the presence of agranular vesicles in all parts of these cells, including the dendrites, somata, axon hillock and axonal initial segment. In all but the last of these sites the vesicles are associated with the 'reciprocal' synapses, which apparently mediate synaptic action, both on to the mitral cells, and away from them, on to the granule cells (Rall et al. 1966); and the presence of the vesicles, especially in such large numbers, is almost certainly due to this unusual synaptic arrangement. It is interesting to ask, however, whether there may be similar mechanisms at similar sites in other sensory systems. With the visual system a very close analogy can be made, for in the retina there are also reciprocal synapses between the secondary neurons of the visual pathway, the bipolar cells and the amacrine cells, which occupy a position analogous to that of the granule cells in the olfactory bulb (Dowling & Boycott, 1966). Although such a clear analogy cannot be made with the other sensory systems, it may be noted that there are vesicles in the neuronal processes which contact the taste bud receptors (Gray & Watkins, 1965) and the cochlea hair cells (Smith & Sjöstrand, 1961). Even more interesting in this regard are the somatodendritic synapses which have been found in the optic tectum of the frog (Sétáló & Székely, 1967) and the apparent dendrodendritic synapses which Lund (1969) has described in the superior colliculus of the rat. In the latter site, processes which resemble dendrites in almost every morphological detail, and which can be traced back to the cell body, have been found to be pre-synaptic to another dendrite, as well as post-synaptic to axon terminals. Similar dendrite-like profiles which receive synapses from axon terminals but which contain vesicles and may be presynaptic to dendritic profiles have also been found in the ventrobasal thalamic nucleus (Ralston & Herman, 1969), the dorsal horn of the spinal cord (Ralston, 1968) and the lateral geniculate nucleus (Guillery, 1969). In the ventrobasal and medial geniculate nuclei of the thalamus, however, presynaptic profiles which occupy analogous positions to these 'dendrite-like' profiles have been found to arise as branches of larger profiles which resemble axon hillocks or initial segments (Jones & Powell, 1969b). While the synaptic organization at these sites is far from clear, therefore, it is worthy of note that all of these sites are important relay stations in various sensory systems.

The presence of synaptic vesicles throughout the mitral cells again raises the
question of where such vesicles originate. It has been suggested that synaptic vesicles are produced by the Golgi apparatus within the perikaryon and then migrate down the axon, but this hypothesis has been criticized on the grounds that vesicles are seldom seen in non-terminal portions of axons (see Whittaker & Gray, 1962). Vesicles are found in the initial segments of mitral cell axons, however, and from our material they do not appear to be related to synapses at this site. As in other neurons, vesicles resembling synaptic vesicles are present in the perikarya of mitral cells, closely associated with the Golgi apparatus; and, in addition, small clusters of agranular endoplasmic reticulum are found in the mitral cell dendrites, which are also closely associated with vesicles (Andres, 1965). These clusters of agranular endoplasmic reticulum resemble portions of Golgi apparatus, and it might be suggested that they develop from the Golgi apparatus and migrate out into the dendrites. Vesicles might then be produced either in the Golgi apparatus itself, or in the endoplasmic sacs in the dendrites, and carried to the more distal parts of the dendrites by cytoplasmic flow. However, although this attractively neat hypothesis might explain other points, such as the accumulation of vesicles in the distal portions of the mitral cell dendrites and similar phenomena in the granule cells of the olfactory bulb, e.g. the presence of vesicles in the spines of these cells (Price & Powell, 1970a), it must be noted that morphological similarity is not sufficient proof that the vesicles associated with the Golgi apparatus or with the endoplasmic sacs are the same as synaptic vesicles.

The mitral cell dendrites can be divided into 2 distinct and spatially separated portions: the dendritic tufts within the glomeruli, which receive synapses from the olfactory nerves and also from the periglomerular cells, and the dendritic shafts within the external plexiform layer, which are synaptically related only to the granule cells through the reciprocal synapses. This situation would seem to be ideally suited to the mechanism of local dendritic integration of the type recently postulated by Diamond and Yasargil on the basis of results obtained in the motoneurons of the fish spinal cord (Diamond, Gray & Yasargil, 1969; Diamond & Yasargil, 1969). A specialized portion of the dendritic apparatus of these motoneurons has been found which receives the excitatory input to the cell from the ipsilateral Mauthner cell, and also part of the crossed inhibitory input; this specialized region appears to be partially insulated from the rest of the cell, both spatially and electrically. This insulation blocks out the background synaptic noise from the rest of the cell and allows local integration of the various influences, with very precise temporal discrimination. Diamond & Yasargil (1969) suggest that there may be a similar mechanism operating in the distal portions of long dendrites in the mammalian central nervous system; in these regions there could be a local integration of excitatory and inhibitory inputs which would act prior to the final integration at the soma-initial segment region. A prerequisite for such a mechanism, however, may be that the region of local integration must be capable of initiating a spike, for otherwise the impulse would be dissipated before it reached the soma-initial segment. The specialized region on the fish motoneuron does seem to be capable of spike generation, and there is evidence that the dendrites of some mammalian neurons may also be able to conduct a propagated impulse (see Diamond & Yasargil, 1969). From the evidence which is available on the
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mitral cell dendrites, however, it is not possible to decide whether or not impulses are actively conducted in the processes (Rall & Shepherd, 1968).

Relatively little can be said about the short axon cells except to emphasize their presence in the granule cell layer of the olfactory bulb; as Cajal reported, these cells are probably not a homogeneous population, but rather have several varieties, the connexions and functions of which may not be the same. By a process of elimination, it has previously been concluded that the axon terminals of at least some of the short axon cells contain relatively small flattened vesicles and form symmetrical (Gray's type II) synapses with the granule cells (Price & Powell, 1970b); if current theories on the morphological characteristics of excitatory and inhibitory synapses are correct (see Gray, 1969), it may be suggested that some, at least, of the short axon cells are inhibitory neurons. Nothing is known about the afferent connexions of these cells except that both spheroidal-vesicle asymmetrical synapses and flattened-vesicle symmetrical synapses have been found on their cell bodies and basal dendrites and that there is experimental evidence in the rabbit to indicate that some, at least, of the asymmetrical synapses are made by axon collaterals of mitral and tufted cells (A. J. Pinching, personal communication). It is possible, however, that they may be the second type of interneuron postulated by Yamamoto et al. (1963) which would be activated by fibres of the anterior commissure, and would also be inhibitory to the granule cells.

With the electron microscope it has been possible to identify and describe all the processes of the granule cells (Price & Powell, 1970a), and, for the most part, those of the mitral cells; the synapses on to these cells have also been analysed (Price & Powell, 1970b, c). Apart from the short axon cells, therefore, a relatively complete description of the neuronal organization of the external plexiform and granule cell layers of the olfactory bulb can now be given. Although most of these connexions have been discussed in previous papers it may be useful to summarize them here; this description is necessarily speculative in some respects, but it synthesizes the results of previous workers and those obtained by our investigation.

The second order neurons of the olfactory pathway, the mitral cells, have primary and secondary dendrites, both of which are found in the external plexiform layer, but only the primary dendrites enter the glomerular layer and receive synaptic contacts from the olfactory nerves. The axons of these cells pass deeply through the granule cell layer, and give off axon collaterals which ramify in that layer, and in the external plexiform layer. In the granule cell layer are situated the granule cells, the deep dendrites of which extend towards the periventricular layer, while the peripheral processes pass superficially into the external plexiform layer, and the short axon cells whose dendrites are confined to the granule cell layer, but whose axons, like the axon collaterals of the mitral cells, may ramify in both the granule cell layer and the external plexiform layer (Cajal, 1911). The distal portions of the peripheral processes of the granule cells carry spine-like gemmules, and these participate in reciprocal synapses with the dendrites and somata of the mitral (and tufted) cells (Hirata, 1964; Andres, 1965; Rall et al. 1966). Cajal considered that the gemmules contacted only the secondary dendrites of the mitral cells, but in the present study
reciprocal synapses have been found on all the dendrites of these cells, regardless of size or orientation, so it is probable that the primary dendrites are also in synaptic contact with the granule cells. This is in agreement with earlier observations by Andres (1965). All the synapses upon the mitral cells, except for those within the glomeruli, have been found to be reciprocal synapses, and it would therefore appear that, outside the glomeruli, these cells may be synaptically influenced only by the granule cells.

The reciprocal synapses are also the only synapses oriented away from the granule cells, but these cells receive synapses from a variety of sources. From outside the olfactory bulb, the centrifugal fibres, fibres of the anterior commissure and collaterals from the ipsilateral anterior olfactory nucleus all terminate upon specific portions of these cells, as do fibres of intrabulbar origin, probably the axon collaterals of the mitral cells and the axons of the short axon cells (Price & Powell, 1970b, c).

The basic neuronal organization of the external plexiform and granule cell layers is therefore relatively simple. All of the extrinsic afferent fibres from the cerebral hemisphere appear to terminate upon the granule cells, which then influence the activity of the mitral cells through the reciprocal synapses. The mitral cells can also influence the granule cells, either through the reciprocal synapses or through the axon collaterals; these 2 pathways need not overlap, as the axon collaterals may act upon granule cells at some distance from the parent mitral cell (small lesions in the olfactory bulb produce degeneration in much of the bulb (Lohman & Mentink, 1969)). However, the presence of the short axon cells complicates the picture considerably; not only would they presumably alter the response of the granule cells to activation from extrinsic afferent fibres and from the mitral cells, but they are themselves subjected to as yet unknown but probably complex influences (possibly including the anterior commissure). Furthermore, a considerable amount of integration between the different afferent inputs must occur within the granule cells, and this is probably determined by the quantitative as well as spatial relationships between the various pathways ending upon the granule cells. As yet, little or no quantitative information is available.

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REFERENCES


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ABBREVIATIONS ON PLATES

a  asymmetrical synapse
ax initial segment of axon of mitral cell
d  dendrite
dp primary dendrite of mitral cell
ds secondary dendrite of mitral cell
gr granule cell
m  mitral cell
n  Nissl granule
og oligodendroglia
s  symmetrical synapse
su short axon cell

Fig. 3. Cell body and basal dendrite of a mitral cell. × 4250.
Fig. 4. Reciprocal synapse (arrows) between a gemmule of a granule cell and the perikaryon of a mitral cell. Note the presynaptic flattened sac (arrowhead) in the cytoplasm of the mitral cell, which is associated with the asymmetrical part of the reciprocal synapse. × 30000.
Fig. 5. Subsurface cistern (arrow) in soma of a mitral cell; note its association with vesicles and ribosomes. × 30000.
Fig. 6. Organelle composed of concentric lamellae which is occasionally found in the dendrites of mitral cells. × 20000.
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Fig. 7. Typical appearance of the dendrites of mitral cells in a coronal section of the external plexiform layer; the section is oriented in such a way that the mitral cell layer would be below this field and the glomeruli above it. The primary dendrites are cut longitudinally and are oriented vertically, while the secondary dendrites are oriented more or less horizontally and are cut in various planes of section. × 11000.

Fig. 8. Secondary dendrite of a mitral cell near its tip; note its varicose outline and the high density of vesicles. × 12000.
Cells of the olfactory bulb
Fig. 9. Initial segment of the axon of a mitral cell. × 21,000.

Fig. 10. Higher magnification of a part of the initial segment shown in Fig. 9; note spheroidal synaptic-type vesicles (big arrow), dense-core vesicles (arrowhead) and alveolate vesicles (small arrows), one of which is fused with the plasma membrane. × 40,000.

Fig. 11. Another example of an initial segment of a mitral cell axon with the same 3 types of vesicles. × 38,000.
Cells of the olfactory bulb
Fig. 12. Cell soma of a short axon cell (middle) together with that of a granule cell (lower left) and of an oligodendroglia (upper left). × 9400.

Figs. 13, 14. Symmetrical (Fig. 13) and asymmetrical (Fig. 14) synapses on to a dendrite which could be traced back to the perikaryon of a short axon cell. × 25000.