THE NEURON TYPES OF THE GLOMERULAR LAYER OF THE OLFACTORY BULB

A. J. PINCHING AND T. P. S. POWELL

Department of Human Anatomy, Oxford, England

SUMMARY

The neurons of the glomerular layer of the rat olfactory bulb have been studied using Nissl staining and Golgi-Kopsch impregnation in light microscopy to define the size, shape and morphological features of individual cell somata, dendrites and axons; these have been correlated with electron-microscopic material in which fine-structural characteristics were also noted for each cell type, particularly synaptic specializations. Three neuron types are described: the external tufted and periglomerular cells of classical microscopy, and additional, superficial short-axon cells; a description of the glomerular arborizations of the mitral and deep tufted cells is also included. The tufted and mitral cells show large, non-spiny glomerular dendritic arborizations, having terminal varicosities, the external tufted cells being more limited in their branching than the deeper cells. External tufted cells have large somata and abundant cytoplasm containing stacks of Nissl material; their main dendrites are characterized by pale cytoplasm and a regular array of neurotubules. Reciprocal dendro-dendritic and somato-dendritic synapses are commonly found, the tufted/mitral cells containing spherical vesicles and contacting by means of asymmetrical membrane thickenings; the other profile involved is a gemmule containing large flattened vesicles and associated with a symmetrical thickening. The periglomerular cells are smaller, with a spiny glomerular arborization, as well as some other dendrites; all the dendrites of these cells tend to be of irregular outline. They have a dark nucleus and very little somatic cytoplasm; somatic and dendritic appendages are common and often contain large flattened vesicles. Synapses oriented from the dendritic shaft or gemmule also show such vesicles, invariably associated with symmetrical thickenings. The superficial short-axon cells are characterized by the entirely periglomerular distribution of their dendrites, which are varicose and rarely branch. Of intermediate soma dimensions, but containing dispersed Nissl material, these cells and their stem dendrites show no regions that can be designated as presynaptic.

Features of axon initial segments, axo-somatic and axo-dendritic synapses are also described for each cell, as well as some unusual glial relationships. Reasons are adduced for relating the superficial short-axon cell to the axon terminal type containing small flattened vesicles, as well as for considering that the external tufted and periglomerular cells show the same synaptic specializations at their axon terminals as at their dendritic and somatic synapses. The cells of the glomerular layer are compared with those of the deeper layers of the bulb and atypical synaptic specializations discussed; some physiological implications of these findings are considered.

INTRODUCTION

The histology of the olfactory bulb at the light-microscopic level has been known in considerable detail for many years, and particularly since the work of Cajal and his contemporaries (Cajal, 1890, 1911, 1955; Van Gehuchten & Martin, 1891; Blanes, 1898). This structure presents, by virtue of its clear lamination, a most suitable site for the study of the peripheral level of a sensory pathway; moreover its accessibility in
certain species and its well known afferent pathways make it a very convenient preparation for physiological study. Such a functional analysis can only be achieved in full, however, with a suitably detailed map of anatomical connexions in the region, both intrinsic and extrinsic; by defining the principal connexions, it should be possible to exclude some of the possible alternative interpretations of electrophysiological recordings. Apart from the study by Andres (1965) on the structure of the whole olfactory bulb, using the electron microscope, other workers have tended to concentrate on certain particular features such as the reciprocal synapses characteristic of this site (Hirata, 1964; Rall, Shepherd, Reese & Brightman, 1966; Price, 1968; Hinds, 1970) or on details of particular cells (Price & Powell, 1970a, b, c, d). The latter authors have investigated the connexions of the mitral, granule and deep short-axon cells of the rat olfactory bulb, as well as demonstrating the mode of termination of the centrifugal pathways to the deep layers of the bulb. The present study, also on the rat, covers the glomerular layer, the site of synaptic contact between the olfactory nerves and the mitral cell and other dendrites; the layer may be divided into 2 major components - the glomeruli and the periglomerular region (Pinching, 1970). This report is concerned with the morphological and synaptic features of the somata of the cells lying in the periglomerular region and of the large mitral cell primary dendrites, as well as those parts of the dendrites and axons of these cells that may be seen in continuity with the cell somata (or primary dendrite) with the electron microscope; the light-microscopic features of these cells are also defined. In the following papers (Pinching & Powell, 1971a, b) the neuropil of the glomeruli and the periglomerular regions is described and the various profiles in these regions identified according to their cells of origin, on the basis of criteria established here.

**MATERIAL AND METHODS**

**Light microscopy**

Several sets of sections of paraffin-embedded Nissl-stained material were available for study; these were stained with thionin and cut at 25 μm. For Golgi impregnations, normal rats, aged between 4 and 7 weeks, were used; the basic Golgi-Kopsch technique (Colonnier, 1964) was used with 2 slight modifications. 1) Fixation was by perfusion using the same method as for electron microscopy (see below) and the bulbs were then immersed in 2% potassium dichromate solution containing 5% glutaraldehyde for 5 days; after this time, they were rinsed carefully in distilled water, and then immersed in 0.75% silver nitrate for a further 5 days. In some cases, this procedure was repeated twice with intervals of 2–3 days in each solution and with careful rinsing between each step. 2) Fixation was carried out by perfusing the animal with a solution of 24% potassium dichromate + 5% glutaraldehyde, after a brief buffered-saline wash-out, followed by immersion in the same solution for 5 days, rinsing and immersing in 0.75% silver nitrate as above. This material was always given a repeated impregnation schedule twice at intervals of 2–3 days in each solution. The second method gave more reliable and regular impregnation, particularly of axons, and generally succeeded in staining a larger number of cells at the glomerular level. Blocks were dehydrated after staining and embedded in low-viscosity nitrocellulose. Sections were cut in the sagittal and coronal planes at 75–100 μm.
**Olfactory bulb: glomerular layer neurons**

**Electron microscopy**

Normal and experimental rats were perfused under Nembutal anaesthesia and hypothermia (15-25 °C) with a mixture of glutaraldehyde (1 %) and formaldehyde (4 %) in 0.1 M phosphate buffer at pH 7.3, following a brief wash-out with buffered salt solution. After some hours, the brain was removed from the cranial cavity and immersed in fixative for a minimum of 3 h; blocks of olfactory bulb were taken and washed in phosphate buffer with 10 % sucrose for periods ranging from a few minutes to overnight. The blocks were post-fixed with osmium tetroxide (2 %), dehydrated in ethanol and embedded in Araldite. Orientation of blocks for electron microscopy was achieved using a 1-2 μm section, stained by the method of Richardson, Jarett & Finke (1960), and ultrathin sections were then cut and stained with uranyl acetate and lead citrate on Formvar-coated slit grids (for serial sections) or uncoated mesh grids. The smallness of many of the profiles in the glomerular layer made serial sections a significant and imperative part of this study, and large numbers of series were used for the greater part of the routine analysis of material. Two major classes of serial sections were used: the first had average dimensions of 500 x 500 μm and each uninterrupted series consisted of 50-120 sections; the second type were about 100 x 50 μm and series of between 100 and 200 sections were obtained, uninterrupted.

**RESULTS**

**Classical histology**

To clarify this study on the glomerular layer, it may be useful first to outline in some detail the concept of this layer held by the classical histologists, particularly the Madrid group. This is not to replace the reading of the valuable and highly detailed original descriptions, particularly those by Cajal (1890, 1911, 1955) and Blanes (1898), which provide authoritative and careful light-microscopic descriptions, but rather to link these with our own Golgi material, which has been studied with a view to correlation with electron-microscopic data.

The olfactory receptor axons, after passing through the cribiform plate of the ethmoid bone, form a layer on the surface of the olfactory bulb from which they penetrate into the subjacent layer of glomeruli; their ramifications within the glomeruli are precisely delineated by the boundaries of these structures and never extend beyond them. Each nerve fibre 'breaks up successively into short, flexuose, relatively stout and extremely varicose secondary branches, and always terminates freely with an olive-shaped or rounded knot' (Cajal, 1890). The same glomeruli also contain the thick expansions of the mitral and tufted cell primary dendrites, which break up on entering the glomeruli into a dense tuft or tree-like arborization; the branches which make up this tuft are varicose and are distributed solely within the glomeruli. There also arborized within these structures the dendrites of the periglomerular cells, which were studied in great detail by Blanes (1898), who described their branches as 'fine and varicose, but not spiny. Some nevertheless exhibit hair-like expansions, that is to say, coverings of relatively long appendages sprouting at a sharp angle.' The glomeruli are surrounded by the cell bodies of the external tufted and periglomerular neurons and of glia, and it is in these interglomerular spaces that the axon terminals and collaterals, other than those of olfactory nerves, terminate; these spaces are now termed the periglomerular region (Pinching, 1970) or 'stratum granulosum externum' (Andres, 1965). The small periglomerular cell bodies are the commonest type, some appearing...
to lie on the very edges of the glomeruli, and others deep in the interglomerular spaces, and they give dendritic arborizations to one or more glomeruli; some lie very close to the external tufted cells, which are also found in this region, particularly in the deeper parts of it. The axons of the external tufted cells distribute collaterals to the neighbouring parts of the glomerular layer, generally terminating in the periglomerular region, and send their main axon deep into the bulb and towards the cerebral hemispheres; the periglomerular cells send all their axonal branches to the periglomerular region around several adjacent glomeruli. The collaterals of the mitral and deep tufted cells do not appear to reach the glomerular layer.

The work of Cajal and his group thus showed the existence of 2 neuron types intrinsic to the glomerular layer – the external tufted cells and the superficial granule cells. The electron-microscopic study described here confirms the existence of the external tufted cells, cells analogous to mitral cells, as well as that of many smaller granule-like, or periglomerular cells, lying in between the glomeruli; in addition, a third type of neuron intrinsic to the glomerular layer has been distinguished, which has features distinct from the other two (Fig. 7). The ability to differentiate a third neuron type with the electron microscope made it necessary to identify such a cell in Golgi-impregnated material; a third type has indeed been characterized (Fig. 4, p. 320) and its light-microscopic features have been confirmed in turn by further electron-microscopic evidence.

The nomenclature of the cells of the olfactory bulb has somewhat altered with the passage of time and it may be useful to clarify the terms at this juncture. Cajal (although his practice varied from time to time) used the designation external tufted cell for those tufted cells lying just deep to or within the glomerular layer, middle tufted for those in the superficial two-thirds of the external plexiform layer and internal tufted for those just superficial to the mitral cells. External or superficial granule cells have more recently become known as periglomerular cells; to avoid confusion with the granule cells of the deeper layers, only this latter term will be used here, despite its looseness. To the third type we have assigned the term ‘superficial short-axon cells’; in addition to its descriptive value, this brings them into line with the third type of the deeper layers described by Golgi, Cajal, Blanes and Van Gehuchten, with which they appear to be analogous.

**Tufted cells**

**Light microscopy.** As far as can be determined, the internal tufted cells entirely correspond with the mitral cells, apart from their more superficial position and smaller size (18–25 μm), and they will not be further considered here. The middle and external tufted cells show a further gradation of size with position, becoming smaller the closer they are to the surface of the bulb; the external tufted cell bodies have a mean diameter of 10–15 μm while the middle tufted are intermediate between these and the mitral and internal tufted cells, according to position. The external tufted cells, which are fusiform or ovoid, lie in the periglomerular region, most commonly in its deeper parts; the middle tufted cells, lying in the external plexiform layer, tend not to be as elongated as the external tufted cells, but still retain an essentially oval
form. In Nissl-stained sections, the somata of these more superficial tufted cells (external and middle) appear similar to the mitral and internal tufted cells showing marked Nissl substance, which is particularly noticeable in the apices of these cells, although the amount varies according to size. Their nuclei are generally pale and

Fig. 1. Camera lucida drawings of neurons impregnated with the Golgi-Kopsch method. A, B, External tufted cells with glomerular arborizations which are more limited than those of mitral cells, but do show varicosities. Axons are marked a and cell A also shows the beginning of a second stem dendrite.
Fig. 2. Camera lucida drawings of neurons impregnated with the Golgi-Kopsch method. A, Glomerular arborization of a mitral cell primary dendrite showing extensive ramification and varicosity of terminal dendrites. B, Periglomerular cell with spiny glomerular arborization and only partially impregnated axon (a).
have a thin fringe of chromatin material and a small nucleolus; their perikarya are relatively large.

In Golgi–Kopsch preparations (Fig. 1), the external tufted cells are seen to have a large primary dendrite which may enter the glomerulus from any angle, depending on the situation of the cell body in relation to its glomerulus, and which branches repeatedly within it. Only rarely do the external tufted cells show secondary dendrites, which, if present, lie in the superficial part of the external plexiform layer or the deep part of the periglomerular region; the middle tufted cells have secondary dendrites arranged in a similar manner to those of the mitral and internal tufted cells. The arborizations of the primary dendrites of the external tufted cells are generally simpler and less tortuous than those of the mitral or deep tufted cells and the branches arise at a more acute angle than those of the latter; in this way they tend to fill a triangular segment of the glomerulus, situated opposite the site of entrance of the primary dendrite (compare Figs. 1 and 2A). In most other respects they are similar to the mitral cell arborizations (see below): the dendrites become increasingly varicose as they branch and decrease in size; they do not show typical spines, although some of the terminal branches may at times take on a spine-like morphology (Fig. 1A). The axons of the external tufted cells are fine, emerging from the soma or initial part of a dendrite, and after pursuing a tortuous course in the periglomerular region and giving off several collaterals to this layer, they pass into the external plexiform layer; they may show a few beadings at irregular intervals.

The glomerular arborizations of the mitral and deep tufted cells are very characteristic (Fig. 2A). Studies at high magnification show that their branches are more profuse and have a greater tendency to be varicose, particularly the terminal branches, than those of the external tufted cells; therefore the arborizations of these deep cells form a dense network which extends over almost the whole glomerulus. This seems to be also partly due to the wider angle of branching of their dendrites so that, instead of the rather fan-shaped arborization of the external tufted cells (Fig. 1), these take on a more spherical outline (Fig. 2A).

Electron microscopy. The tufted cells are characterized by their large size relative to other somata at the same depth, the cytoplasmic abundance evident at their apices, and marked granular endoplasmic reticulum (Figs. 7–9). In view of the uneven distribution of the cytoplasm around the nucleus, identification on the basis of cytoplasmic volume alone is insufficient, since several cells are cut through their centres, at right angles to the axis of the oval; also there may be little endoplasmic reticulum in the thin portions of the cytoplasm (Fig. 8). The tufted cells may be readily distinguished with the electron microscope, however, by virtue of the reciprocal synapses commonly found on their cell somata and dendrites (Figs. 9, 18, 19), similar to those on mitral cells (Hirata, 1964; Andres, 1965; Rall et al. 1966; Price & Powell, 1970d); this primary criterion has been used for defining equivocal cell somata and thus serial sections have been an invaluable aid (Figs. 18, 19).

The nuclei of tufted cells are pale relative to those of periglomerular cells but the nuclear material stands out more clearly than that of short-axon cells and often has a patched appearance (Fig. 7); they may show slight nuclear indentations, commonly
opposite the base of the primary dendrite, but these are rarely deep or complex (Fig. 8). The nucleoli are well delineated and show a clear distinction into granular and fibrillar components; a very fine layer of chromatin surrounds the nucleus, giving these cells a very characteristic appearance at low magnifications.

The cytoplasm of the external tufted and middle tufted cells is pale, but rich in granular endoplasmic reticulum and this is most commonly found as multiple parallel stacks in the apices of the cell somata (Fig. 9); large complexes of Golgi apparatus are characteristic, notably at the bases of dendrites and sometimes quite distant from the cell soma (Fig. 20). Ribosomal rosettes are common and are especially concentrated in the nuclear indentations. In the larger tufted cells, some of the mitochondria may present a slightly blown up appearance (Fig. 8); several large dense lysosomes and lipoprotein bodies are very often present in the cytoplasm of these cells, although rarely present in such numbers in other cell types. Some cells of this type are surrounded by several thin glial lamellae, similar to those observed on their dendrites (Fig. 19).

The main dendritic shafts of the tufted cells are smooth in outline and of wide diameter, and have a characteristically pale appearance resembling the wider primary dendrites of the mitral and internal tufted cells; they arise from one or both sides of the cell soma and appear to be drawn out from them (Fig. 8). The neurotubules of the tufted cell dendrites are typically very regularly arranged. These dendrites show many reciprocal synapses with gemmules of granule cell peripheral processes or of periglomerular cell dendrites, in the external plexiform and glomerular layers respectively. One or more thin glial lamellae may cover the surface of the dendrites in the periglomerular region and superficial part of the external plexiform layer, except at regions of synaptic contact, and this covering usually remains more or less intact until their entry into the glomeruli (Figs. 16, 17). The origin of these lamellae is certainly glial and has been unequivocally determined in many cases by noting continuity with obvious glial profiles containing filaments or glycogen granules or with the glial soma (Fig. 17); nevertheless some periglomerular cell processes may also sometimes become extended into thin sheets (Reese & Brightman, 1970). On occasion the same glial cell has been seen to ensheath bundles of olfactory nerve fibres at the surface and to form typical glial lamellae around the dendrites of mitral or tufted cells in the periglomerular region (Fig. 17). These probably correspond with the glial cells of the olfactory nerve layer described by Blanes (1898) as having stout, granular peripheral expansions and several long smooth and unbranched fibres that pass centrally in between the glomeruli; the latter are clearly demonstrated by the Weigert method and may well be responsible for the formation, in the monkey, of myelin around this dendritic segment (Pinching, 1971). It is possible, however, that the interglomerular glial cells that Blanes describes also contribute to these lamellae.

At the point of entry into the glomeruli the tufted cell dendrites branch, and around this region of primary glomerular branching, characteristic unidirectional symmetrical synapses are found on the dendrites from definite axon terminals containing large flattened vesicles (Fig. 20); these terminals may be identified as being those of periglomerular cell axons (see below). Reciprocal synapses with periglomerular cell gem-
mules are also found on this periglomerular part of the dendrite. The reciprocal synapses, which the tufted cells show on their somata and dendrites are exactly comparable to those of the mitral cells (Figs. 9, 18, 19). In the external plexiform layer these synapses are formed with the gemmules arising from the peripheral processes of granule cells, which are present up to the deep edge of the glomerular layer; in this region and more superficially the gemmules of periglomerular cells are also found and participate in reciprocal synapses with the tufted cells and their processes, the synapses showing the same polarities as those with the granule cells. The tufted cells always show an aggregation of spherical vesicles in their dendrites or somata at the points of synaptic contact that are directed away from the profile and the membrane thickenings are always of the asymmetrical type (Figs. 9, 18); the gemmules, on the other hand, regularly contain flattened vesicles of the large type (Price & Powell, 1970b) and synapse back on to the tufted cell with symmetrical membrane thickenings (Figs. 9, 19). These polarities are a constant feature of the reciprocal synapses, wherever they occur, and have been confirmed by serial sections. Flat sacs, occupying half or the whole of the presynaptic position, have also been noted on the tufted cell side of the reciprocal synapses in the somata (Fig. 18) or large dendrites, similar to those described for mitral cells (Hirata, 1964; Price & Powell, 1970d). Tufted cell somata and stem dendrites very rarely receive any synapses other than those from gemmules and from the axon terminals of periglomerular cells; occasionally, however, asymmetrical synapses are found on the cell somata or stem dendrites from axon terminals containing spherical vesicles (Pinching & Powell, 1971b). In the unusual instance of a tufted cell soma that extends partially across the border between the glomerular and periglomerular regions, it may receive an asymmetrical synapse from an olfactory nerve terminal, but these terminals are not found in the periglomerular region (Pinching, 1970).

Occasionally somato-somatic and dendro-somatic synapses have been observed to occur between external tufted cells and periglomerular cells (Fig. 24); the synaptic specializations oriented from the tufted cell soma or dendrite are the same as those of the tufted cell half of the reciprocal synapse with gemmules and have spherical vesicles and an asymmetrical thickening. A slight 'symmetrical' membrane thickening may be found adjacent to this type of synapse polarized in the opposite direction, with a flat sac of smooth endoplasmic reticulum on the periglomerular cell side (Fig. 25), similar to that described by Hirata (1964) and Price & Powell (1970d). It is noteworthy that most of the somato-somatic contacts of this sort occur on, or close to, the axon hillock of the tufted cell involved. The regular presence of spherical vesicles in the cell somata (Figs. 18, 24) and dendrites of tufted cells in relation to synaptic specializations strongly suggests, by the morphological corollary of Dale's principle (Eccles, 1964), that all regions of these cells involved in synaptic transmission will have such spherical vesicles, notably the axon terminals; similarly, the regularly asymmetrical membrane specializations of dendro-dendritic and somato-somatic synapses may be considered as a constant feature of all the processes of these cells.

The axon hillock of tufted cells (Fig. 8) is characterized by an absence of Golgi apparatus and rough endoplasmic reticulum, as well as by the presence of several
neurotubules, often aggregated. The latter pass into the initial segment of the axon which shows typical features of this region (Palay, Sotelo, Peters & Orkand, 1968; Peters, Proskauer & Kaiserman-Abramof, 1968; Westrum, 1970), that is, aggregated tubules, many free ribosomes, plasma-membrane undercoating and dense material in the surrounding extracellular space; dense-cored vesicles, smooth agranular vesicles and alveolate vesicles are also common, the latter often budding off the plasma membrane. These axon initial segments may emerge from any part of the cell soma of the tufted cells or occasionally from the initial part of their dendrite. Synapses on to the axon initial segments are rare but, when present, they are always of the symmetrical type, the presynaptic vesicles of the axon terminal being the large flattened type characteristic of periglomerular cells; reciprocal synapses with gemmules are found on initial segments of middle tufted cells, and this constitutes a difference from the mitral cells (Price & Powell, 1970), but all tufted cells are similar to mitral in that reciprocal synapses also occur on the axon hillock.

The large, smoothly outlined mitral and deep tufted cell dendrites (Fig. 16) pass from the external plexiform layer, through the deep aspect of the periglomerular region and into the glomeruli; their pale cytoplasm is very characteristic and the neurotubules are very regularly arranged throughout the dendritic profile. They have reciprocal synapses with the gemmules of the granule and periglomerular cells, in the external plexiform and glomerular layers respectively; the polarities and characteristics of these are exactly similar to those described for the deeper parts of the mitral cells by Price & Powell (1970), and for the tufted cells above. Like the dendrites of the tufted cells, these dendrites are often covered by several thin glial lamellae, particularly as they pass through the superficial part of the external plexiform layer and periglomerular region (Fig. 16). Around their point of primary glomerular branching they receive symmetrical synapses from periglomerular cell axon terminals containing large flattened vesicles, but do not give a return synapse; very occasionally the shaft of the mitral cell primary dendrite may receive an asymmetrical synapse in the periglomerular region from an axon terminal containing spherical vesicles. These dendrites also make asymmetrical dendro-somatic synapses with periglomerular cell somata (Fig. 23).

Periglomerular cells

*Light microscopy.* These spherical or occasionally ovoid cells surround the glomeruli together with the other glomerular layer neurons and many glial cells of similar Nissl appearance; the periglomerular cells are also similar in this material to the granule cells of the deeper layers. They range in diameter from 5 to 8 μm and have dark nuclei with a clumped fringe of chromatin and relatively large, dark and uneven nucleoli; their perikarya are thin and unstained. Golgi-impregnated material (Figs. 2A, 3, 5) shows that these cells may give rise to dendritic arborizations in more than one glomerulus, as noted by Blanes (1898), though one field may greatly exceed the others in extent (Fig. 3A, B); they may also have short dendrites which extend into the periglomerular region (Fig. 3B, C). Their glomerular arborizations rarely fill the glomeruli
Fig. 3. Camera lucida drawings of neurons impregnated with the Golgi-Kopsch method. Periglomerular cells: A shows spiny glomerular arborization and axon stub; B shows typical length of axon, but has 2 limited glomerular arborizations, somatic spines and periglomerular dendrites; C demonstrates an irregular glomerular arborization and a short beaded axon.
but seem to contribute in most cases to a circumscribed part of them, similar to those of the tufted cells. Of those that arborize within the glomeruli many show very varicose secondary and tertiary branches, from which spring a large number of spine-like appendages of variable shapes and sizes and which may have very long pedicles (Figs. 2B, 3A); it is considered that these long-pedicle appendages are the ‘hair-like appendages’ of Blanes (1898), as they often appear to have no head or else this head is so far from the dendritic shaft that it may seem separate. Other cells show very irregular, rather than varicose, dendrites, both within and between the glomeruli, which are more like glial processes, although these too may branch and give rise to some spines in the glomeruli (Fig. 3B, c). Somatic spines (Fig. 3B) are regularly seen, as are spines on the initial parts of dendrites.

The axons of the periglomerular cells are not always evident in Golgi-stained material, even after repeated impregnations, a fact also noted by Blanes, who considered this to be an impregnation defect. In view of the difficulty of staining these cells at all, and indeed of impregnating the axons of any of the cell types of the glomerular layer, such an explanation would seem fair; however, the absence of an axon in the granule cells, to which the periglomerular cells are in many ways analogous, suggests the possibility that some periglomerular cells may be entirely lacking in an axonal process. Serial electron-microscopic sections have always revealed an axonal process, tending to rule out this possibility, but the inevitably low sampling prevents a final judgment on this point. Some cells show a short stub of axon in Golgi material which probably corresponds to the initial unmyelinated segment of axon (Fig. 3A); when it has been possible to trace the axon for greater distances, it has rarely reached beyond 3 or 4 glomeruli and shows much beading at irregular intervals (Fig. 3B, c). These periglomerular cell axons may occasionally branch and their course is predominantly in the periglomerular region.

**Electron microscopy.** Periglomerular cells are the commonest neuron type in the glomerular layer but may appear similar to some glial cells (Fig. 7); for this reason, only those cells that show synaptic specializations can be certainly identified as periglomerular cells. Their dark nuclei almost fill the cell somata, leaving a very thin layer of cytoplasm (Fig. 10) which only expands opposite the origins of the cell processes. The chromatin at the edge of the nucleus is markedly clumped into large and uneven masses which extend deeply into the nucleus, where they generally merge with the nucleolus; nuclear rodlets are occasionally found (Fig. 24), but are small and ill-defined, being very much rarer in the rat than in the rabbit (see Siegesmund, Dutta & Fox, 1964; Estable-Puig & Estable-Puig, 1970). The bulk of the nucleus has an irregular, patchy appearance, as a result of considerable local clumping of the nuclear material; at several points deep indentations may extend into the nucleus, which may be quite complex (Fig. 10). The thin cytoplasmic shell surrounding the nucleus is darker than that of other neurons and contains many ribosomes arranged into rosettes, but very little granular endoplasmic reticulum. Most of the perikaryal organelles are concentrated around the bases of the dendrites where a few segments of endoplasmic reticulum may be seen, rarely organized into stacks, but regularly surrounded by a particularly large number of ribosomal rosettes; large Golgi complexes and most of
the somatic mitochondria are encountered in these regions. On some occasions, the periglomerular cell soma may be enveloped in a glial wrapping of one or more thin lamellae, which only stop at the sites of emergence of the cell's processes; these have been noted by Brightman (1968) and differ from those of neuronal origin, also observed in this study, described by Reese & Brightman (1970).

The dendrites are of 2 broad types: large, pale dendrites (Fig. 22) and thin, darker ones; both have a rather uneven outline, particularly those of the thinner class, giving them an appearance similar to, but never as marked as, that of glial processes. Cytoplasmic organelles extend for a considerable distance into the large periglomerular cell dendrites, and Golgi apparatus is regularly seen at the base of these dendrites though less commonly in relation to the thin dendrites. The bases of the thin dendrites are more commonly associated with endoplasmic reticulum and many free ribosomes, the latter extending well into the dendritic shafts in many cases. Neurotubules are irregularly disposed and flexuose, notably those of the thinner dendrites; they are widely spread in the large dendrites but seem more concentrated in the thinner ones. These latter processes may become extended into thin sheets of cytoplasm, without tubules, similar to glial sheets (see Reese & Brightman, 1970), and may partially enclose a tufted or mitral cell dendrite, from which they may receive an asymmetrical synapse (see Pinching & Powell, 1971b); no return synapses from these sheets have been seen. Many spines arise from the cell soma and the large dendrites of these cells and have variable appearances: most somatic spines are short or sessile protrusions with a characteristically grey or flocculent cytoplasm and may contain a few cisternae, probably representing spine apparatus, and a number of vesicles (Figs. 11, 12). Some somatic and most dendritic spines are larger with longer pedicles, and often contain many large flattened vesicles, a few cisternae, mitochondria and a variable number of ribosomes, free or as rosettes. The vesicles in the somatic and some of the proximal dendritic appendages are not related to synaptic contacts directed away from the periglomerular cell; a similar finding has been reported for the spines of the granule cell deep dendrites (Price & Powell, 1970a).

Somatic and dendritic spines of periglomerular cells most commonly receive asymmetrical synapses from pale axon terminals containing spherical vesicles, which are never olfactory nerve terminals (Fig. 11); synapses may on occasion be from a mitral or tufted cell dendrite or a tufted cell soma. The bases of these spines and the heads of others may also receive symmetrical synapses from axon terminals containing flattened vesicles, either of the large or small type (Fig. 12), and from gemmules (see Pinching & Powell, 1971a) of periglomerular cells containing large flattened vesicles. These 2 axon terminal types showing symmetrical membrane thickenings frequently synapse on to the cell somata or dendritic shafts of periglomerular cells, the terminals with small flattened vesicles predominating, and are a very common feature of profiles of this type of cell (Figs. 10, 12); asymmetrical synapses from axon terminals containing spherical vesicles are seen less frequently on main dendritic shafts or cell somata, but are more common on the finer dendritic shafts (Pinching & Powell, 1971b). The cell soma and either type of dendrite may, however, receive an asymmetrical synapse from a tufted cell soma or a mitral or tufted cell primary dendrite, as described
in the previous section (Figs. 23, 24), but a sac and limited membrane thickening oriented away from the periglomerular cell soma are the only possible synaptic structures that have been seen indicative of a reciprocal synapse (Fig. 25); a similar sac and membrane thickening may be associated with the region of contact between a periglomerular cell soma and an adjacent terminal containing spherical vesicles – probably a tufted cell recurrent collateral. Such sacs have been seen in continuity with granular endoplasmic reticulum (Fig. 25) and are similar to those found opposite one half of the tufted or mitral cell component of the reciprocal synapses on their cell somata (Price & Powell, 1970b, d). Extracellular material is present in the intercellular cleft similar to that found in relation to more typical synaptic structures; no vesicles have been seen in relation to this apparent synaptic structure, despite the fact that vesicles are regularly found in somatic spines (Fig. 11). The large periglomerular cell dendrites may make synaptic contact from their shafts (Fig. 22) or by means of a spine-like appendage, the gemmule, with the primary dendrites of mitral or tufted cells; these synapses are regularly characterized by symmetrical membrane thickenings and large flattened presynaptic vesicles. The somato-somatic, somato-dendritic, and dendro-dendritic synapses (Figs. 22–24) in the 2 directions between the somata or primary dendrites of mitral or tufted and periglomerular cells are not always reciprocal in the immediate vicinity of each synaptic specialization, although the reverse synapse may occur between the processes of the same 2 cells at some distance (see Pinching & Powell, 1971a).

The initial segments of the periglomerular cell axons are difficult to identify as they show few of the typical characteristics of these processes; this may be a feature of small neurons (Fig. 21). The initial segments are thin, of fairly regular outline and contain a few neurotubules, which only rarely appear to be aggregated and are never markedly so. Many free ribosomes are present, however, and limited plasma membrane undercoating may occasionally be discerned; multivesicular bodies, dense-cored vesicles and coated vesicles, and flattened agranular vesicles are common, and seem characteristic of this type of axon initial segment. Synaptic contacts are regularly found on these structures, usually 2 or 3 per process; they are always of the symmetrical type, though the presynaptic terminal may contain either small or large flattened vesicles (Fig. 21). Small irregular cisternae may be found to lie in the immediate post-synaptic cytoplasm, probably analogous to the cisternal organ in the initial segments of other cells (Peters et al. 1968; Westrum, 1970; Kemp & Powell, 1971). The axon hillock is identifiable in the periglomerular cells by the absence of Golgi apparatus and the presence of a limited amount of granular endoplasmic reticulum and many ribosomes, free or in rosettes; this region is not generally very marked since the axon initial segments of these cells appear to arise abruptly from the soma surface.

The regular presence of vesicles of the large flattened type (see Price & Powell, 1970b) in the somatic and dendritic spines (Fig. 11), main dendritic shafts (Fig. 22) and their gemmules and axon initial segments of periglomerular cells is strong evidence that this type of vesicle is to be found in all parts of these cells, particularly those related to synaptic contacts – i.e. the finer dendritic branches in the glomeruli and
their gemmules, and the axon terminals of these cells; similarly, the symmetrical membrane thickening at synaptic contacts appears to be a regular feature of synapses oriented from periglomerular cells.

**Short-axon cells**

*Light microscopy.* In Nissl-stained material the short-axon cells may be discerned in the periglomerular region as spherical or slightly ovoid, but have larger somata with a more irregular outline than the periglomerular cells. They also have characteristically pale nuclei with a very thin fringe of chromatin and a small rounded nucleolus; their perikaryon is larger than that of the periglomerular cells but shows much less Nissl substance than that of tufted cells. These cells are less common than the other two neurons of the glomerular layer, and resemble the short-axon cells of the granule cell layer, although they are rather smaller, being 8–12 μm in diameter. In Golgi-impregnated material (Figs. 4, 6) they are particularly distinguished by their dendritic field, which is entirely periglomerular, the dendrites weaving in between the glomeruli or lying deep to them (Fig. 4 A, D); others seem to surround a single glomerulus with a clasp-like formation of dendrites (Fig. 4 B, C); the dendrites of the superficial short-axon cells are characteristically stout. They branch very infrequently, unlike the other glomerular layer neurons, have a typically varicose or irregular outline (Fig. 4 B, C), and, while some show dendritic and somatic spines (Fig. 4 A, C, D) others seem free of spines; these differences may reflect a further subdivision of dendritic types similar to that described for the short-axon cells of the deeper layers. The axons of the superficial short-axon cells, when they are stained, are short, on average shorter than those of the periglomerular cells (Fig. 4 C, D): they rarely traverse more than 1 or 2 glomeruli, and pass around or through the edges of these structures; they tend to be beaded but show little branching.

*Electron microscopy.* The superficial short-axon cells, which are less common than periglomerular or external tufted cells, may be found lying at any point around the glomeruli in the periglomerular region; they are predominately spherical and appear of the same order of size as the external tufted cells (Fig. 7). They are characterized by pale nuclei and a moderate amount of cytoplasm, intermediate between the other 2 types, which is fairly evenly distributed around the nuclei (Figs. 7, 13). The nuclear material is irregular, but less formed in patches than that of tufted cells (Fig. 7), and the chromatin around the edge is thin; nucleoli are well defined. Nuclear indentations are common and may be deep and quite complex, containing many ribosomes and some granular endoplasmic reticulum. The granular reticulum in the bulk of the cytoplasm is arranged into small groups of 2 or 3 parallel stacks and never forms the large complexes typical of the tufted and mitral cells. Golgi apparatus is found at the bases of dendrites, while ribosomes, mitochondria and other typical neuronal inclusions are frequent throughout the cytoplasm. The dendrites of the superficial short-axon cells are large and many cytoplasmic organelles may be found in their initial parts; neurotubules often appear rather irregularly disposed in the dendritic cytoplasm, apparently in relation to surface irregularities and varicosities, unlike the well ordered array of tubules in the smooth dendrites of tufted and mitral cells. The short-axon
Fig. 4. Camera lucida drawings of neurons impregnated with the Golgi-Kopsch method. Short-axon cells A and D distribute their dendrites to the periglomerular region adjoining several glomeruli, while B and C have dendrites forming a 'clasp' around a single glomerulus. A, C and D show a few spines and the dendrites of B and C are notably varicose; C and D show characteristic lengths of axon. Note that C is at a lower magnification than the other 3.
cell dendrites are always directed into the periglomerular region, in which they may follow tortuous paths, and are of irregular shape, becoming increasingly varicose at a distance from the cell soma, as is seen in Golgi-impregnated material. Spines are seen on cell somata and dendrites, but are rare compared with those of periglomerular cells; they are characteristically small and contain a few irregular cisternae in a flocculent cytoplasm, and sometimes small mitochondria.

Although these cells sometimes appear similar to external tufted cells, they may be readily distinguished by the absence of reciprocal synapses on their cell body and dendrites, as confirmed by serial sections; in order to ascertain this fact, several series of consecutive sections were examined, in which the whole cell soma and part of the dendrites could be observed (Fig. 13). Each series consisted of between 100 and 200 sections, and each short-axon cell, after tentative identification as such, was rigorously examined for synaptic contacts and other distinguishing features. Agranular vesicles were never found in the cell somata or somatic spines of these cells; another criterion for identification that emerged from studies of long series of sections is the common appearance of asymmetrical synaptic specializations directly on to the cell soma, from pale axon terminals containing spherical vesicles (Figs. 14, 15). Direct synapses of the asymmetrical kind on to the cell somata of the other 2 cell types are very rare, but are characteristic of the superficial short-axon cells. Symmetrical synaptic contacts are also commonly found on these cell somata and main dendrites and are made by axon terminals containing either small or large flattened vesicles. The main dendritic shafts receive all synaptic types found in the periglomerular region (thus excluding olfactory nerve terminals), and by far the most common are the asymmetrical type; the spines arising from cell somata and dendrites also receive synapses from this latter type of terminal. Dendrites of short-axon cells contain few, if any, vesicles, and these are irregular structures, and are never aggregated or related to synaptic structures off the dendrites.

An unusual and characteristic feature of the short-axon cell somata, and to a lesser extent of their dendrites, is the presence of small cytoplasmic protrusions around synaptic terminals of the asymmetrical thickening type; these may seem at some points to encircle the terminal, but they have never been observed to receive a synapse from it. The cytoplasm of these protrusions is typically flocculent but is otherwise empty of organelles. It is possible that these structures bear some relationship to spines, in view of their similar cytoplasm and the fact that asymmetrical terminals characteristically terminate on to the somata of the other cell types of this region by means of somatic spines; it may therefore be necessary to include them in the broadening concept of spine types (Jones & Powell, 1969), as a stage less than sessile.

The axon hillocks of short-axon cells are not at all marked in the majority of cases and show little, if any, funnelling of cytoplasm (Fig. 13); where present, they contain granular endoplasmic reticulum and many ribosomes, but no Golgi apparatus. The initial segment regularly emerges abruptly from the cell soma (Fig. 13). It shows the typical features of this region, the aggregation of neurotubules and coating of the plasma membrane being very marked; dense-cored, alveolate and agranular vesicles are common, the latter showing considerable variety. Synapses are found on these...
Discussion

In this paper the cell types of the glomerular layer of the olfactory bulb have been described from observations with Nissl staining, Golgi impregnation and electron microscopy. It has been shown that, in addition to the 2 neuron types intrinsic to this layer described by the classical histologists—the external tufted and periglomerular cells, a third type may be recognized—the superficial short-axon cell. These 3 types have been characterized according to several morphological criteria with each method, notably electron microscopy, with a view not only to distinguishing the cell bodies and initial parts of their processes but also to providing means of identifying these processes at a distance from the somata, when demonstration of continuity with an identifiable cell is not possible. Although this study has been carried out on the rat, and the descriptions and figures in this and the following papers have been drawn exclusively from this species, the bulbs of the rabbit and to a lesser extent the cat and monkey have also been examined; as far as can be assessed, these bulbs are similar to that of the rat but with certain differences in general morphology (Pinching, 1971); further slight differences may emerge, but the broad organizational principles appear to be the same in the four species.

The discovery of a third cell type in the glomerular layer and its subsequent confirmation with several techniques is a clear demonstration of the value of correlative studies with electron microscopy and Golgi impregnation. The additional detail provided by electron-microscopic examination strongly suggested the existence of a third neuron type at this level, and light-microscopic studies confirmed the existence of a distinct cell type which might correspond to it; the Golgi-impregnation characteristics of this neuron further aided its electron-microscopic characterization and particularly that of its processes. The relative rarity of these cells, together with the difficulty of impregnating cells in the glomerular layer, would not have permitted their identification using the Golgi technique alone, as evidenced by their neglect in the careful and meticulous works of Cajal and Blanes and other classical histologists. However, some illustrations, notably by Golgi (1875), who considered them to be glial, and by Van Gehuchten & Martin (1891) as well as some of those by Blanes (1898), indicate that these authors had seen such cells but had insufficient evidence to distinguish them from glial or periglomerular cells. Furthermore, the possibility that short-axon cells may develop later than the other types (Hinds, 1968) would have tended to militate against their being regularly observed by the Madrid histologists, who both preferred to use very young animals. Nevertheless, a personal study by one of us (A. J. P.) of some of Cajal’s original preparations (in the Cajal Museum of the Institute Cajal, Madrid) did reveal some cells of this type in preparations from older animals. It is felt therefore that the superficial short-axon cells were not described by these histologists for reasons of rarity and an obvious caution against the possibilities of incomplete impregnation or of false identification of glia (the latter was already a
source of contention in the bulb as regards the granule cells). In view of the very strong electron-microscopic evidence that is now available, as well as considerable Golgi evidence for the existence of an additional cell type, we feel justified in putting forward the superficial short-axon cell as a definitive neuron type of this layer.

The presence of a short-axon cell in the glomerular layer has greatly extended the already significant parallels between this and the deeper layers, comprising the granule and mitral cell layers (equivalent to the periglomerular region) and the external plexiform layer (broadly equivalent to the glomeruli), so that each of the intrinsic neurons of the glomerular layer has a counterpart with similar features in the deeper layers. Thus the external tufted cells seem analogous to the mitral and deep tufted cells, the periglomerular cells to the granule cells and the superficial short-axon cells to the deep short-axon cells. Each glomerular neuron shows a broadly similar morphology to its deep counterpart, but is generally smaller and shows a different orientation of its processes - glomerular or non-glomerular - rather than predominantly vertical or horizontal. The type, density and relative proportions of nucleus and cytoplasm and the distribution of organelles within them are strikingly similar in the analogous cell types of the 2 groups of layers (see Price & Powell, 1970a, d). Although there is no overlap between the distribution of processes of granule and periglomerular cells, nor of the 2 short-axon cell types, each pair being limited by the deep aspect of the glomerular layer, the tufted and mitral cells represent more of an overlapping continuum from superficial to deep, both receiving glomerular activity in their terminal dendritic tufts.

Certain notable differences between the cells of the 2 groups do exist, however. The granule cell (Price & Powell, 1970a) has no axon, but rather 2 types of dendritic processes - the deep dendrites, which are varicose and give rise to typical spines, and the peripheral processes that give rise to some spines and to gemmules which enter into reciprocal synapses. The periglomerular cell on the other hand does possess an axon, but its dendritic arborization within the glomeruli appears to be analogous to the granule cell peripheral processes in bearing some spines and many gemmules. The periglomerular cells may also show a more glial-like dendritic process having an irregular outline, which often becomes drawn out into thin sheets, of which the granule cell appears to have no counterpart. The analogy between the peripheral process of the granule cell and the large glomerular dendrites of the periglomerular cell is further strengthened by the regular presence of Golgi apparatus in the cell soma at the bases of these processes and not opposite the axon or glial-type dendrites of the periglomerular cell or the deep dendrites of the granule cell (Price & Powell, 1970a). The nature of the dendritic processes in the olfactory bulb will be further considered in a later paper (Pinching & Powell, 1971b).

The external tufted cells have various special characteristics in which they differ from mitral and deep tufted cells - these are the extent of their glomerular arborizations, their relative lack of secondary dendrites and the additional distribution of their collaterals. It has already been noted that the glomerular arborizations of the external tufted cells are characteristically wedge-shaped, with the result that they cover only a portion of the glomerulus, while the deep tufted cells and mitral cells have arboriza-
tions that fill the glomeruli with an almost spherical distribution. Such a difference could be related to the fact that the mitral and the tufted cells of the external plexiform layer always enter from the deep aspect of the glomeruli; by extending throughout the glomeruli they subserve, these cells must receive afferent impulses from olfactory nerves terminating in all parts of the glomerulus, while a given glomerulus is 'covered' by several external tufted cells whose dendrites arise from different points around it. It is interesting to note in this respect that Land, Eager & Shepherd (1970) show that degeneration of olfactory terminals within the glomeruli after localized lesions of the nerves, may be restricted to part of a single glomerulus. Secondly, the external tufted cells are unusual in that they rarely have secondary dendrites, only those cells on the edges of the external plexiform layer ever showing them, although one cell may show two primary dendrites, each with a separate glomerular arborization. The fact that the secondary dendrites of mitral and deep tufted cells are related exclusively to the reciprocal synaptic contacts with granule cell gemmules (Price & Powell, 1970d) indicates that these secondary dendrites are specialized to establish such contacts; their absence in many of the external tufted cells further supports this view, since the primary dendrites of these cells are able to establish a considerable number of similar contacts with the periglomerular cells, the glomerular analogues of the granule cells, by the nature of the glomerular formations. The distribution of axon collaterals of the tufted cells to the glomerular layer is a further difference between them and the deep cells, which distribute collaterals only in the granule cell and external plexiform layers (Cajal, 1955; Valverde, 1965). Thus, in spite of the many striking similarities between mitral and tufted cells, the latter and particularly the external tufted cells, do form a distinct group in the extent and levels of their connexions; the observation of reciprocal synapses on the initial segments of middle tufted cells, indicates that these too may form a partially separate group.

The concept, introduced by Valverde (1965), that the tufted cells are inwardly displaced periglomerular cells rather than outwardly displaced mitral cells (as Cajal considered) has not been borne out by this electron-microscopic study: no periglomerular-type neurons have been observed in the external plexiform layer, while the characteristic tufted neurons lying in this layer are all of the mitral type in general cytological features and particularly in the possession of reciprocal synaptic arrangements with granule cell gemmules, showing the same types of polarity as exhibited by the mitral cells. Similar cells, the external tufted cells, in the periglomerular region have also been demonstrated to show mitral-type features; Cajal's classification of the mitral and tufted cells in all layers of the olfactory bulb as a single basic group has thus been confirmed at electron-microscopic level and is a striking tribute to his insight.

Three types of vesicles may be distinguished in normal aldehyde-fixed material of the glomerular layer of the bulb as in the deeper layers – spherical vesicles, and large and small flattened vesicles (see Price & Powell, 1970b). It should be noted that for the brains in which flattening was less marked, probably for reasons of varying osmolarity of fixative, processes could be identified by the greater irregularity of shape shown by the flattened-type vesicles as compared with the regular spherical type. The presence of synaptic vesicles related to membrane thickenings in the cell bodies
and dendrites of the mitral and tufted cells and in the dendrites of periglomerular cells as well as that of exactly similar vesicles in the spines of the latter, has considerably aided the identification of vesicle-containing profiles at a distance (Pinching & Powell, 1971a, b); large flattened vesicles are characteristic of periglomerular cells and their processes, like the granule cells, while spherical vesicles are found in the processes of mitral and tufted cells. Axon terminals containing small flattened vesicles are also found in the glomerular layer and this type of terminal, recognized by site and mode of termination, only degenerates after intrinsic lesions of the glomerular layer (Pinching & Powell, unpublished observations) but no dendrites have been found containing this type of vesicle. Since the distribution of these small flattened vesicle axon terminals corresponds to that of the superficial short-axon cell axon known from Golgi impregnation evidence, they may be identified as being those of the short-axon cells by a process of elimination. A similar line of reasoning has identified the terminals containing small flattened vesicles in the deeper layers as being those of the short-axon cells of the granule cell layer (Price & Powell, 1970a, b).

The observation of somato-somatic, dendro-somatic, and dendro-dendritic synapses between cell types identifiable by their somata has assisted the identification of the processes involved in similar synaptic arrangements on the distal dendrites in the neuropil. Such synapses are only found between mitral or tufted and periglomerular cells and the synaptic membrane thickenings are characteristic – those from mitral and tufted cells being regularly asymmetrical and those directed from periglomerular cells being symmetrical. It can therefore be deduced that dendrites showing such polarities in reciprocal or serial synaptic arrangements are those of the same cells. Such an observation, to which no exceptions have been found, would confirm an analogy between the periglomerular cell dendrites and granule cell peripheral processes and of the tufted cells with the mitral cells. On this basis we cannot agree with the interpretation of Hinds (1970) as to the polarities of the reciprocal synapses. The false interpretation stems from his identification of the cell shown in fig. 5 of his report as a periglomerular cell, whereas in our consideration there is little doubt, even from the small part of the cell body shown, that it is an external tufted cell, by virtue of its general cytology and its synaptic specialization; indeed many exactly similar arrangements have been observed in this study in which the neuronal soma involved was always identifiable as an external tufted cell, while no such relationships are found in the case of periglomerular cells.

The presence of vesicles in the dendrites of many of the cells of the olfactory bulb and their presynaptic position has been known for some time (Hirata, 1964; Andres, 1965; Rall et al. 1966; Price, 1968; Price & Powell, 1970a–d) and although initially considered to be a peculiarity of this region, it is emerging that neither the presence of vesicles nor a presynaptic role are the entire preserve of axon terminals in several other parts of the central nervous system (e.g. Dowling & Boycott, 1966; Sétáló & Székely, 1967; Lund, 1969; Ralston & Herman, 1969; Famiglietti, 1970). Certainly in the olfactory bulb, where the majority of profiles contain vesicles, it can be difficult on occasion to know whether one is dealing with axons or dendrites; in the glomeruli, the position has been greatly simplified by knowledge obtained from past and present.
Golgi studies on the distribution of axons and dendrites in this layer (see Pinching & Powell, 1971a). Criteria have to be established nevertheless by which vesicle-containing dendrites and axon terminals may be distinguished. The distribution of vesicles—homogeneous or otherwise—and the presence of ribosomes or endoplasmic reticulum may partially fulfil this requirement, but at present there is little substitute for the examination of large numbers of serial sections to demonstrate continuity with an unequivocal dendritic shaft or length or axon. If vesicle-containing and presynaptic dendrites are shown to be widespread in the nervous system, then some anatomical and physiological work may need re-interpretation in the light of such a demonstration. There is no doubt that the presence of synaptic specializations oriented away from dendrites of otherwise typical neurons, notably those with axons such as the mitral, tufted and periglomerular cells, suggests that such a peculiarity is not solely a feature of cells that lack axonal processes, like the granule and amacrine cells of the bulb and retina. However, it could be suggested that these presynaptic dendrites represent a form of cell process that is intermediate between axon and dendrite, with some properties of each (see Pinching & Powell, 1971b). That such an intermediate process exists could emerge from a physiological examination of these unusual cells in the olfactory bulb; certainly their physiological properties pose a considerable problem and must be evaluated before the exact significance of their participation in the neuronal organization within any nucleus can be assessed. It should be emphasized, however, at this juncture, that although some previous studies have focused upon the unusual aspects of the organization of the olfactory bulb neurons, these aspects exist not in isolation, but in a larger context which includes straightforward axo-dendritic and axo-somatic arrangements; the analysis of the mode of combination of all these features underlies the essential purpose of this study, which is to produce a comprehensive anatomical circuit diagram of the cells of the olfactory bulb.

The large number of vesicle-containing processes which enter into synaptic contact within the glomerular layer has had further repercussions in that it has provided additional evidence for the correlation established by Colonnier (1968) between type of vesicle and type of membrane thickening. It can be stated unreservedly that the correlation between spherical vesicles and asymmetrical contacts, and between flattened vesicles of both types and symmetrical thickenings has been entirely confirmed in every case in which synaptic contacts have been examined. Serial sections have proved invaluable for ensuring that the type of thickening being examined is cut in such a way as to show its genuine characteristics; this is particularly important because the periphery of an asymmetrical thickening often appears to be 'symmetrical'.

The demonstration of 'sac junctions', in which a flat sac, continuous with endoplasmic reticulum lies presynaptic to part or whole of a membrane thickening of either type, extends the distribution of such apparently synaptic structures to the tufted and periglomerular cells, in addition to those already described for the mitral and granule cells (Hirata, 1964; Price & Powell, 1970b, d). Although the synaptic nature of these sac junctions is difficult to ascertain, the similarity of the membrane specializations and the presence of an undoubted layer of extracellular material between the membranes (of the type associated with typical synaptic structures)
suggests that these are specializations of a similar type. The facts that sac junctions usually occur with the appropriate membrane thickening polarities as those of typical dendro-dendritic synapses, and may even share the presynaptic site with vesicles (in the mitral and tufted cells), all indicate that the flat sac of endoplasmic reticulum situated presynaptically may be analogous to, or a substitute for, presynaptic vesicles. Nevertheless, it must be noted that the similar appearance of cisternae opposite persistent post-synaptic membrane thickenings (Pinching, 1969) cannot be taken as evidence of functional contact; indeed the possibility remains that at least some of the above 'sac junctions' are cases in which a degenerating terminal has left behind its post-synaptic structures. The similarity of the 'presynaptic cisternae' in the 2 cases could be taken to suggest that some of these apparent synaptic structures have been left over, developmentally, and the sac or cisterna has been induced opposite it in a similar way to the apposed process after long-term degeneration. These observations on sacs or cisternae in relation to membrane thickenings are clearly impossible to assess on solely anatomical grounds; thus while in some cases they would seem to be functionally related to synaptic specializations (in mitral and tufted cells), their presence in other situations may represent either a form of functional contact of a non-synaptic nature, or else a non-functional synaptic contact. Whether or not the sacs have any relation to the derivation of vesicles cannot be stated on this evidence.

The mitral and tufted cells present a site in which the questions of dendritic integration (Rall et al. 1967; Diamond & Yasargil, 1969) and dendritic spike generation may be studied (Rall & Shepherd, 1968); this point has already been considered in an earlier paper (Price & Powell, 1970) but new observations on the glomerular components of these cells may throw further light upon these problems. The main primary dendrites of the mitral and tufted cells enter the glomeruli by passing through the periglomerular region and then branching several times within the former. Around the point of primary branching and within the periglomerular region these large dendrites are covered by several layers of thin glial lamellae; also in this region they receive the symmetrical synaptic contacts of the periglomerular cell axons, additional to the reciprocal dendro-dendritic synapses characteristic of all but the most distal parts of the mitral and tufted cell dendritic trees. The extensive glomerular arborizations of the stem dendrites receive the massive and excitatory input from the olfactory nerves (Yamamoto, Yamamoto & Iwama, 1963; Shepherd, 1963) in addition to receiving the probably inhibitory dendro-dendritic synapses from periglomerular cells; the point at which the post-synaptic effects of such inputs will converge will thus be at the region of primary glomerular branching. Clearly any inhibition applied at this level, mediated by the periglomerular cell axon terminals, could be highly effective in reducing or preventing any excitatory effect from spreading beyond the glomeruli; similarly, any excitation will have a strong facilitatory effect. If, as is possible (Rall & Shepherd, 1968; see also Spencer & Kandel, 1961), this is a region of dendritic spike generation, similar functionally to the axon initial segment, local inhibition will be powerful in preventing spike initiation. The role of the glial lamellae in relation to dendritic function is less straightforward: it may serve to insulate this critical region of integration from local 'noise' or may perform a role similar to that
of a myelin sheath, leading to some form of facilitated conduction through the peri-
glomerular region; the formation of a myelin sheath around this specific segment of
dendrite in the monkey (Pinching, 1971) may add support to this interpretation.

It is interesting to consider the significance of the probable origin of this glial
wrapping of the large mitral and tufted cell dendrites in view of developing ideas on
a dynamic role of glia in the central nervous system. If, as is strongly indicated, some
of the lamellae originate from deep processes of the glial cells whose peripheral
processes wrap around the bundles of incoming olfactory nerve fibres, then these cells
occupy a position closely related to both input and output of the glomerular relay;
in this way they could be considered similar to the Müller cells of the retina (Miller &
Dowling, 1970), although the contact of the bulbary glia with the neurons is more
intimate. The observation by Cajal (1890) and Blanes (1898) that these cells are stained
by the Weigert method indicates a myelin-like role for their processes, and the observa-
ton on myelination at this site in the monkey further suggests such a role. The further
possibility exists that, like the Müller cells of the retina, these glia act as sinks or
electrodes for K⁺ ions, and may as such play an active role in the relay system in the
glomeruli: thus the peripheral processes of this glial cell type will be subjected to a
large potential change when the olfactory axons in the bundles are active, and this
may be transmitted as a slow glial potential to the large mitral and tufted cells dendrites
as they leave the glomeruli having received synaptic contact from the incoming
olfactory axons. The striking anatomical similarity of the olfactory nerve bundles to
the fibre bundles of the optic nerve of *Necturus*, on which much work on the ionic
properties of neuroglia has been done (see Kuffler & Nicholls, 1966) is certainly sug-
gestive that the anatomical relationship in the two sites may have similar ionic
consequences.

Finally, it should be noted that the somata of the cells of the glomerular layer may
lie very close together or even with large areas of plasma membrane directly apposed;
in addition, the 3 major types are often situated in close proximity to each other
(Fig. 7). These small cells may be difficult to record from singly with present tech-
niques, and potentials recorded in the periglomerular region are likely to be derived
from several cells of different types, operating variously in a single locality. In view
of this and the extensive interconnexions of the various processes detailed here and
in the following papers (Pinching & Powell, 1971 *a*, *b*), electro-physiological studies
should be interpreted with caution; it is hoped that the findings presented in these
papers may allow certain possibilities to be excluded and the remaining interpretations
to be tested.

This work was supported by grants from the Medical and Science Research Councils. We
are most grateful to the Director of the Instituto Cajal, Madrid, for giving one of us (A.J.P.)
the opportunity of studying Cajal's original preparations.
REFERENCES


A. J. Pinching and T. P. S. Powell


(Received 11 March 1971)
Olfactory bulb: glomerular layer neurons

ABBREVIATIONS ON PLATES

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>axon</td>
</tr>
<tr>
<td>c</td>
<td>cell soma</td>
</tr>
<tr>
<td>d</td>
<td>dendrite</td>
</tr>
<tr>
<td>er</td>
<td>granular endoplasmic reticulum</td>
</tr>
<tr>
<td>et</td>
<td>external tufted cell</td>
</tr>
<tr>
<td>f</td>
<td>glial filaments</td>
</tr>
<tr>
<td>g</td>
<td>gemmule</td>
</tr>
<tr>
<td>gl</td>
<td>glia</td>
</tr>
<tr>
<td>go</td>
<td>Golgi apparatus</td>
</tr>
<tr>
<td>is</td>
<td>axon initial segment</td>
</tr>
<tr>
<td>ly</td>
<td>lysosome</td>
</tr>
<tr>
<td>m</td>
<td>mitochondrion</td>
</tr>
<tr>
<td>n</td>
<td>nucleus</td>
</tr>
<tr>
<td>no</td>
<td>nucleolus</td>
</tr>
<tr>
<td>pg</td>
<td>periglomerular cell</td>
</tr>
<tr>
<td>r</td>
<td>intranuclear rodlet</td>
</tr>
<tr>
<td>s</td>
<td>spine</td>
</tr>
<tr>
<td>sa</td>
<td>short-axon cell (superficial)</td>
</tr>
<tr>
<td>sj</td>
<td>sac junction</td>
</tr>
<tr>
<td>t</td>
<td>axon terminal</td>
</tr>
<tr>
<td>u</td>
<td>unmyelinated olfactory nerve fibre</td>
</tr>
<tr>
<td>z</td>
<td>nuclear indentation</td>
</tr>
</tbody>
</table>

Arrow-heads indicate synaptic specializations from the post-synaptic side.
A. J. Pinching and T. P. S. Powell

Fig. 5. Photomicrograph of part of a periglomerular cell impregnated with the Golgi-Kopsch method, showing characteristic appendages. x 1620.

Fig. 6. Photomicrograph of part of a superficial short-axon cell (see Fig. 4c) impregnated with the Golgi-Kopsch method, showing varicose dendrite and axon. x 900.

Fig. 7. Low-magnification electron micrograph of the periglomerular region showing the 3 neuron types of the glomerular layer – external tufted, periglomerular and superficial short-axon cells – as well as a glial cell. x 6600.
Fig. 8. External tufted cell soma showing initial part of dendrite and initial segment of axon. $\times 12\,000$.

Fig. 9. Detail of external tufted cell, showing characteristic stacks of endoplasmic reticulum; note also the 2 components of the reciprocal synapse in relation to 2 different gemmules, and adjacent sac junction. $\times 21\,500$. 
Olfactory bulb: glomerular layer neurons
Fig. 10. Periglomerular cell soma showing marked nuclear indentation and receiving a symmetrical synapse; note paucity and darkness of cytoplasm. × 22000.

Fig. 11. Somatic spine of periglomerular cell, containing large, irregular, flattened-type vesicles, which receive an asymmetrical synaptic contact from an axon terminal containing spherical-type vesicles. Note also evidence of symmetrical contact, outside the plane of this section, from an axon terminal containing small flattened-type vesicles. × 53500.

Fig. 12. Axon terminal containing small flattened vesicles (compare adjacent terminal with spherical vesicles) synapsing with symmetrical thickenings on to a somatic spine and the cell soma of a periglomerular cell. × 53500.
Olfactory bulb: glomerular layer neurons
A. J. Pinching and T. P. S. Powell

Fig. 13. Superficial short-axon cell soma showing several nuclear indentations and axon initial segment. This cell was traced throughout in serial sections to ensure correct identification. × 15,000.

Fig. 14. Asymmetrical synapse on to the soma of the same cell as in Fig. 13, from an axon terminal, containing spherical vesicles, that also contacts a vesicle-filled appendage; the large flattened vesicles of this appendage identify it as deriving from a periglomerular cell. × 50,000.

Fig. 15. Asymmetrical-type synapse on to short-axon cell soma from axon terminal containing spherical vesicles. × 67,000.
Fig. 16. Mitral cell primary dendrite at the point of primary glomerular branching, covered by several thin glial lamellae; the pale swollen profile may be a sheet derived from a periglomerular cell thin dendrite. × 18,000.

Fig. 17. Glial profile giving rise to a thin lamella that surrounds a tufted cell primary dendrite, approximately in the middle of the glomerular layer. The same glial cell also surrounds a bundle of olfactory nerve fibres running from the olfactory nerve layer towards a glomerulus. × 45,200.
Olfactory bulb: glomerular layer neurons
Fig. 18. Part of a series of sections through a reciprocal synapse on an external tufted cell soma. Synaptic specializations oriented from the soma showing spherical vesicles, a flat sac of endoplasmic reticulum and an asymmetrical membrane thickening. See also Fig. 19. × 48,000.

Fig. 19. Further section from same series as Fig. 18, showing the return synapse from the gemmule on to the cell soma; this has large flattened vesicles and a symmetrical membrane thickening. Note glial lamellae passing over the gemmule, which continue over the cell soma. × 48,000.

Fig. 20. Axon terminal of periglomerular cell, containing large flattened vesicles, synapsing with a symmetrical thickening on to the stem dendrite of a tufted cell; no return synapse was found in serial sections, and the profile was continuous with a length of preterminal axon. Golgi apparatus is commonly found in the initial parts of tufted cell dendrites. × 53,500.

Fig. 21. Synapses on to axon initial segment of periglomerular cell, showing large flattened vesicles and symmetrical thickenings. Note that periglomerular cell initial segments show little evidence of neurotubular aggregation, and have limited plasma membrane undercoating. × 40,000.
Olfactory bulb: glomerular layer neurons
Fig. 22. Dendro-dendritic synaptic contact showing large flattened vesicles and symmetrical thickening; the profile making this synapse was traced back to its cell of origin, a periglomerular cell, and the identity of the vesicle type and thickening type confirmed in serial sections. No return synapse was found but the post-synaptic process is probably the dendrite of a mitral or tufted cell. × 53 500.

Fig. 23. Dendro-somatic synapse from the stem dendrite of a mitral cell on to the soma of a periglomerular cell; spherical presynaptic vesicles and asymmetrical membrane thickening. × 53 500.

Fig. 24. Somato-somatic synapse from an external tufted cell soma on to a periglomerular cell soma; spherical presynaptic vesicles and asymmetrical thickening. The periglomerular cell shows an intranuclear rodlet. × 42 800.

Fig. 25. Somato-somatic sac junction from periglomerular cell to external tufted cell; sac of endoplasmic reticulum situated 'presynaptically' and an equivocal membrane thickening, probably symmetrical in comparison with adjacent profiles. × 53 500.
Olfactory bulb: glomerular layer neurons