J. Anat. (1983), **137**, 1, pp. 1–19
With 11 *figures*
Printed in Great Britain

The re-innervation of olfactory glomeruli following transection of primary olfactory axons in the central or peripheral nervous system*

J. R. DOUCETTE†, J. A. KIERNAN AND B. A. FLUMERFELT

Department of Anatomy, The University of Western Ontario, London, Ontario N6A 5C1, Canada

(*Accepted* 28 *September* 1982)

INTRODUCTION

The olfactory receptor cells are located in the epithelium lining the dorsomedial portion of the nasal cavity. Their unmyelinated axons terminate within the olfactory bulb in circumscribed areas of grey matter termed glomeruli (Pinching & Powell, 1971; Shepherd, 1972; Willey, 1973). The olfactory epithelium is the only region of the nervous system in adult mammals in which neurons are regularly replaced (see Takagi, 1971; Graziadei & Monti Graziadei, 1978*a*, 1979). Non-neuronal cells at the base of the olfactory epithelium divide and differentiate into receptor cells. Their axons then grow through the olfactory nerves to the olfactory bulb and terminate within glomeruli. In rodents, the life span of each receptor neuron is approximately five weeks.

It is generally held that transection of the olfactory nerves results in death of the olfactory receptor cells (Oley *et al.* 1975; Simmons, Rafols & Getchell, 1981). The transection also deprives the glomeruli of their primary olfactory afferent fibres. New receptor cells are formed within the olfactory epithelium, and their axons grow through both peripheral and central nervous tissue to reinnervate the glomeruli. (Oley *et al.* 1975; Graziadei & Monti Graziadei, 1976, 1978*b*, 1980; Harding, Graziadei, Monti Graziadei & Margolis, 1977; Monti Graziadei, Kaplan, Bernstein & Graziadei, 1980).

In studies of the re-innervation of the mammalian olfactory bulb, the olfactory nerves have been transected on the intracranial side of the cribriform plate of the ethmoid bone. Here the axons are ensheathed by Schwann cells of the peripheral nervous system. In this study, we examine the re-innervation of glomeruli denervated by cutting their afferent axons within the central nervous system, in the nerve fibre layer of the olfactory bulb. The ensheathing cells here are not Schwann cells but central neuroglia, ultrastructurally similar to the astrocytes of very young animals (Doucette, 1981). The re-innervation of glomeruli denervated by incising the nerve fibre layer is compared with the re-innervation that follows transection of the olfactory nerves at the cribiform plate.

* Reprint requests to Dr J. A. Kiernan.
† Present address: Department of Neuroscience, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada.

MATERIALS AND METHODS

Male albino Wistar rats weighing 200–250 g were used. The animals were anaesthetised, for all operative procedures including perfusion of fixative and killing by decapitation, with intraperitoneally injected pentobarbitone and chloral hydrate (Valenstein, 1961).

This communication is concerned with the olfactory bulbs of the experimental animals. Changes in the olfactory epithelia of some of the rats are the subject of another paper (Doucette, Kiernan & Flumerfelt, 1983).

Normal material

Eleven rats were perfused with formaldehyde–glutaraldehyde solution exactly as described by Palay & Chan-Palay (1974). The olfactory bulbs were exposed and their fixation was continued by immersion in the same fluid for 18 hours at 4 °C. The olfactory bulbs were then removed, cut into sagittal slices approximately 0·5 mm thick, post-fixed in 2 % osmium tetroxide in 0·12 M phosphate buffer containing 7 % dextrose, contrast-stained *en bloc* with uranyl acetate, dehydrated, and embedded in an Epon-Araldite mixture (Anderson & Flumerfelt, 1980). A glass knife was used to cut sections 0·5–1·0 μm thick, which were then stained with 1 % toluidine blue in 1 % borax for optical microscopy. After further trimming of the blocks, ultrathin sections of selected areas were cut with a diamond knife, stained on grids with lead citrate (Venable & Coggeshall, 1965) and examined in an electron microscope.

Transection of olfactory nerves

The right olfactory bulb was exposed by drilling through the frontal bone in 14 rats, and the olfactory nerves were severed by passing the blade of a cataract knife between the bulb and the cribriform plate. We have shown elsewhere (Doucette *et al.* 1983), as have Monti Graziadei & Graziadei (1979), that this procedure transects approximately three quarters of the fascicles that traverse the cribriform plate. Most of the spared nerve fibres enter the ventromedial surface of the olfactory bulb. After operation, the rats were allowed to survive for intervals ranging from 4 to 56 days. Specimens of the olfactory bulbs were then prepared as described above for the normal tissues.

Incision in the olfactory bulb

In 34 rats, the right olfactory bulb was exposed and an incision approximately 0·5 mm deep was made transversely across its dorsal surface. The cut was located 1–2 mm caudal to the rostral pole of the bulb. After intervals from 2 to 183 days, the olfactory bulbs of 27 of the operated animals were prepared for electron microscopy as described above.

In the remaining 7 rats, approximately 1 mg of horseradish peroxidase (HRP; 'Type VI', Sigma Chemical Co., St Louis, Mo., U.S.A.) was applied to the olfactory bulb. In three rats, it was placed on the incision immediately after bleeding had ceased; these rats were killed 24 hours later. The other animals were allowed to survive for 14 or 31 days before re-exposing the olfactory bulb and applying horseradish peroxidase to a second transverse incision. This second incision was made near the rostral pole of the bulb, anterior to the previous lesion. The rats were killed 24 hours later. The olfactory bulbs of the animals to which horseradish peroxidase was applied were processed for histochemical demonstration of peroxidase in serial

sagittal frozen sections. The method of Mesulam (1978), with the minor modifications proposed by Mesulam *et al.* (1980), was followed exactly. With this technique, it was possible to observe anterogradely transported horseradish peroxidase in the distal parts of the severed axons of the nerve fibre layer of the olfactory bulb. The positions of glomeruli labelled with horseradish peroxidase were plotted diagrammatically in relation to the positions of the lesions. This enabled us to compare the normal distribution of glomeruli innervated by axons coursing across the dorsal surface of the olfactory bulb with the distribution two or four weeks after transection of the primary olfactory fibres.

RESULTS

Normal olfactory bulb

The ultrastructure of the normal olfactory bulb has been thoroughly studied (see Shepherd, 1977), so we mention only those features that are important for comparison with the material taken from the experimental animals. The olfactory bulb is a laminated structure in which the primary olfactory afferent axons form the most superficial layer (Fig. 1). Here, the olfactory axons were arranged in bundles. The cytoplasmic processes of the ensheathing neuroglial cells divided the bundles into smaller groups, each cell enclosing 10–50 axons in the same mesaxon. The olfactory axons were unmyelinated, 0·1–0·5 μm in diameter, and contained microtubules, neurofilaments and occasional mitochondria.

Within glomeruli (Fig. 2), the terminal and pre-terminal portions of the olfactory axons were electron-dense and were arranged in groups of varying size. Each group of axons was separated from others by the dendritic processes of mitral, tufted and periglomerular cells. Astrocytic processes were only rarely observed within glomeruli. Olfactory axons were presynaptic to the dendrites within a glomerulus and were never observed to occupy postsynaptic positions. Dendrodendritic synaptic terminals were frequently observed in the glomeruli. It was not possible to determine whether any individual dendrite belonged to a mitral, a tufted, or a periglomerular cell. The larger profiles were assumed to be those of mitral and tufted cells. However, as the dendrites of the latter two types of neuron approach their termination, they become smaller (Pinching & Powell, 1971) and therefore resemble the dendrites of the periglomerular cells. For this reason, dendrites of small diameter could not be assigned to any particular type of neuron.

Degenerating olfactory terminals, which contained swollen and irregularly shaped vesicles, were occasionally found within the glomeruli of unoperated animals. In the glomeruli, the dendrites sometimes contained dense membranous whorls and multivesicular bodies in their cytoplasm. Similar structures have been described in dendrites following partial de-afferentation of the inferior olivary nuclei of adult cats (Walberg, 1963), and in the lateral geniculate nucleus of the monkey following enucleation of an eye (Wisniewski, Ghetti & Horoupian, 1972). They are thought to be derived from degenerated presynaptic terminals that have been phagocytosed by dendrites.

Transection of olfactory nerves at the cribriform plate

By the fourth post-operative day, the glomeruli on the dorsal surface of the olfactory bulb contained degenerating boutons, though some normal ones were also present. The degeneration progressed with time, so that by the 24th day (Fig. 3) there were no normal olfactory axons left in the dorsal part of the bulb, and most of

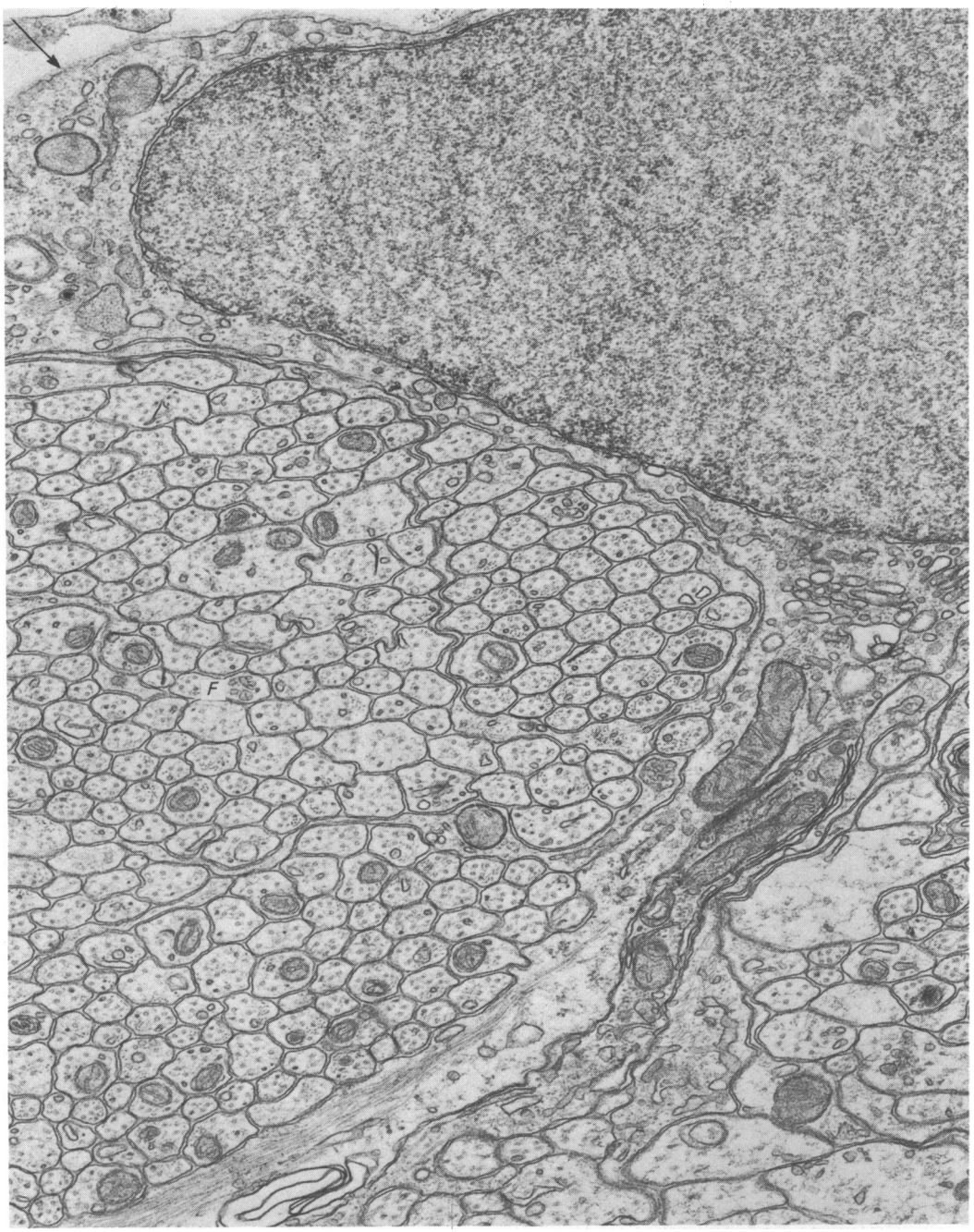


Fig. 1. The nerve fibre layer of a normal rat's olfactory bulb. The nucleus of an astrocyte is present in the top right hand part of the field. This cell forms the basal lamina of the external glial limiting membrane (arrow) and also encircles a fascicle (*F*) of primary olfactory afferent axons. The cytoplasmic processes within the fascicle belong to cells other than the astrocyte. Electron micrograph. ×25500.

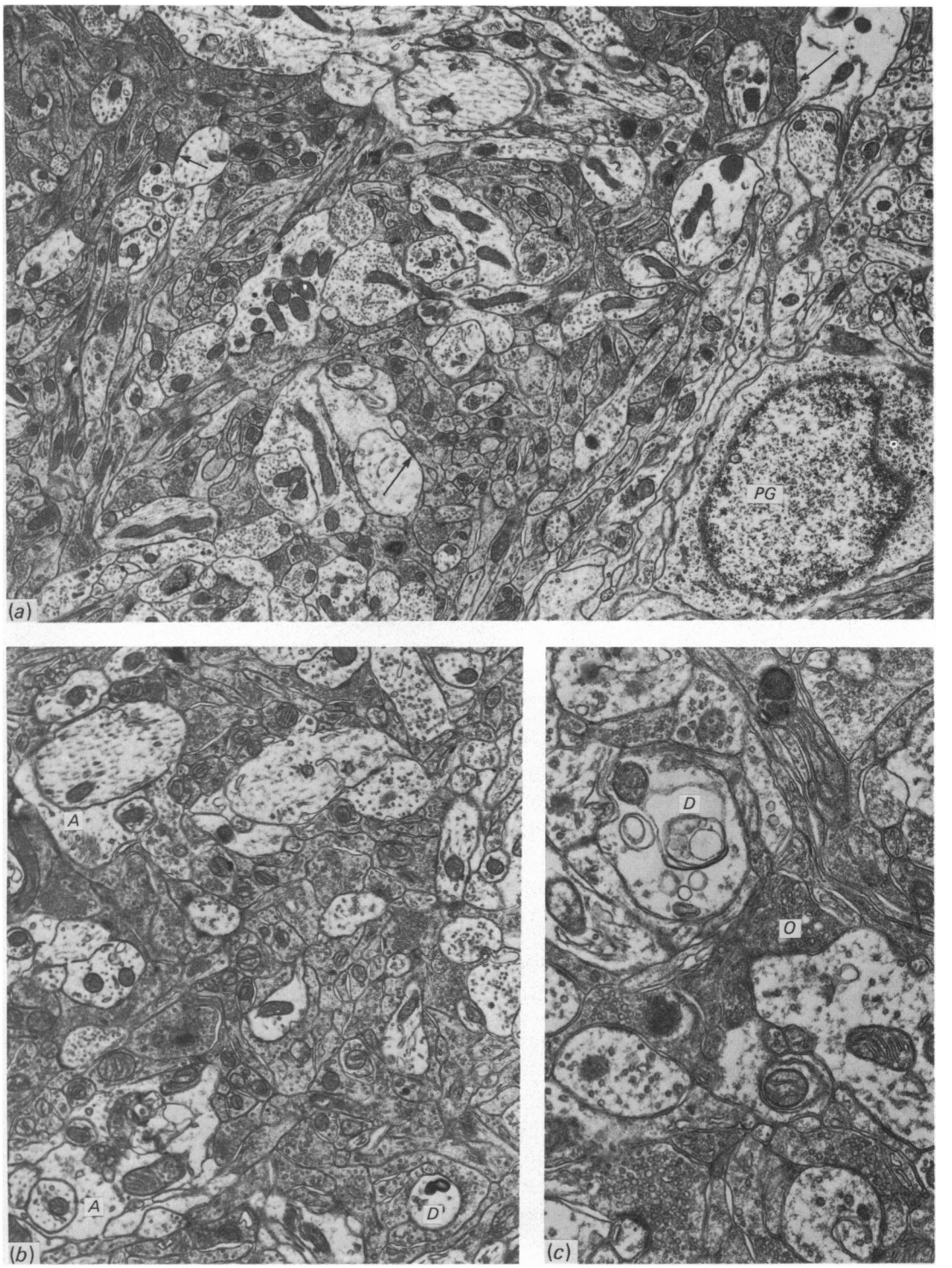


Fig. 2(*a–c*). Glomeruli in a normal rat's olfactory bulb. (*a*) Neuropil near the edge of a glomerulus, and a periglomerular neuron (*PG*). The dark profiles are of primary olfactory axons; the light profiles are of dendrites. Some axodendritic synapses are marked by arrows. Electron micrograph. × 10250. (*b*) Another area of glomerular neuropil, including two astrocytic processes (*A*) and a dendrite containing osmiophilic debris (*D*). Electron micrograph. × 12600. (*c*) A degenerating olfactory axonal terminal (*O*) containing swollen synaptic vesicles, in a normal glomerulus. A dendrite (*D*) contains phagocytosed membranous material. Electron micrograph. × 20550.

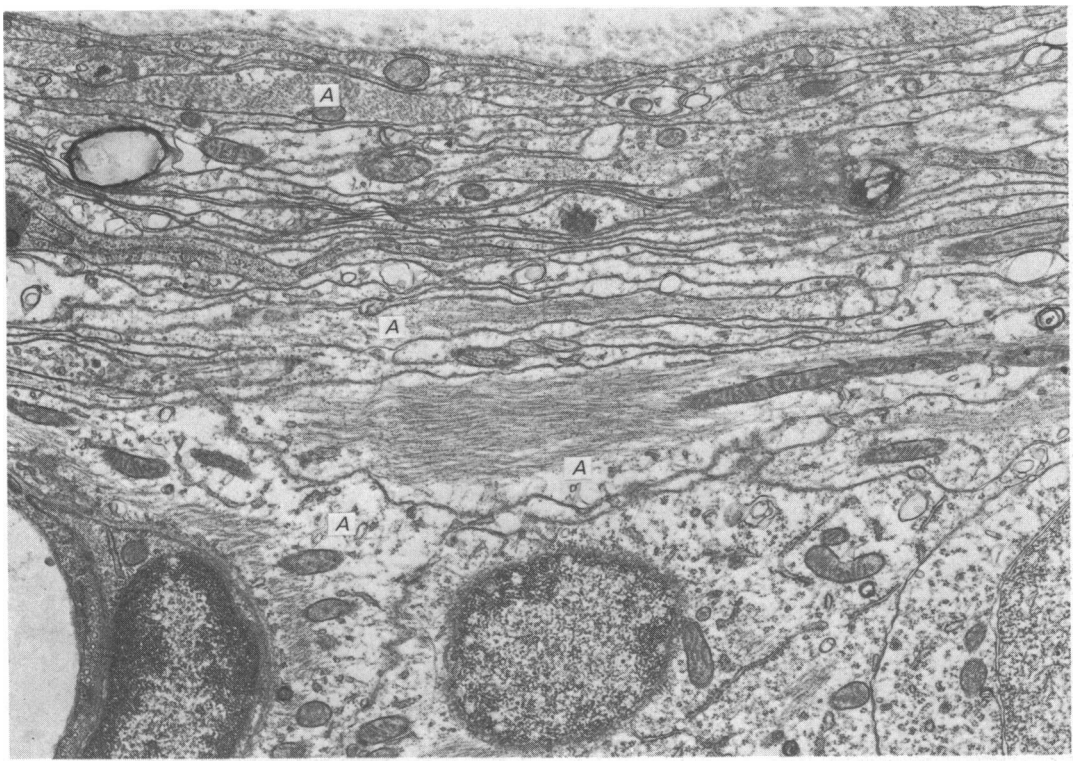


Fig. 3. Nerve fibre layer of the dorsal surface of the olfactory bulb, 24 days after transection of the olfactory nerves at the cribriform plate. Axons are absent and the hypertrophied processes of astrocytes (*A*) predominate. Electron micrograph. × 10700.

the degenerating ones had evidently been phagocytosed. Electron-dense debris indicative of phagocytosis occurred abundantly in the dendrites of the glomeruli at this time and also in cells that had appeared within the glomerular neuropil. These small cells had elongated nuclei, and cytoplasm that contained numerous dense bodies, scanty endoplasmic reticulum and no microtubules, in an electron-dense matrix. They were identified as reactive microglial cells, which are believed to enter abnormal regions of the central nervous system by emigration from blood vessels (see Fujita & Kitamura, 1976; Oehmichen, 1978). The denervated glomeruli also contained the cytoplasmic processes of hypertrophied astrocytes, recognisable by their content of glial filaments.

Re-innervation of the glomeruli occurred gradually after the 24th post-operative day. Although large areas of some glomeruli were still denervated, most glomeruli contained normal numbers of olfactory axons in synaptic contact with dendrites by 42 days (Fig. 4). At this time, and also at 56 days, phagocytic debris was still present in many of the dendrites, and occasional reactive microglial cells were present in some glomeruli. The degeneration and reappearance of axons in the nerve fibre layer paralleled that observed in the glomeruli, but the phagocytic cells were hypertrophied astrocytes, recognisable by virtue of their abundant cytoplasmic filaments.

Site of incision in dorsal surface of olfactory bulb

In all cases, the transversely oriented incision extended through the nerve fibre and glomerular layers into the external plexiform layer, and it usually reached the

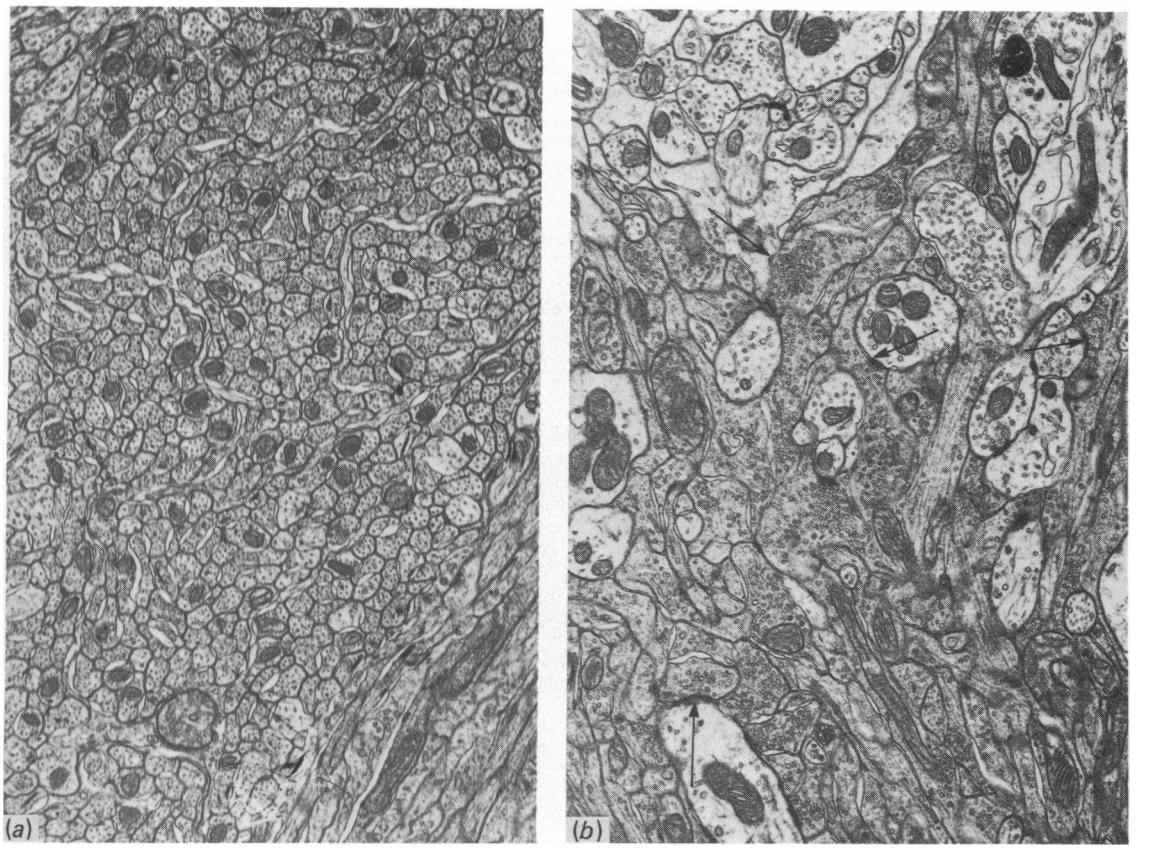


Fig. 4(*a–b*). The olfactory bulb 42 days after transection of the olfactory nerves at the cribriform plate. (*a*) Nerve fibre layer with large bundles of axons. Electron micrograph. × 12650. (*b*) Glomerulus, with olfactory axons (dark), dendrites (light) and some axodendritic synapses (arrows). Electron micrograph. × 10800.

granule cell layer. There was often a slight depression where the knife had penetrated the surface of the bulb, and at this level there was also a dislocation of the mitral cell layer (Fig. 5). Otherwise, the incision did little damage to the structure of the bulb. Even those glomeruli lying in close proximity to the lesion were undistorted. At the site of the lesion (Fig. 6), the astrocytic processes were hypertrophic and contained dense bodies in their cytoplasm. Reactive microglial cells were also present. Connective tissue, continuous with the pia mater, was present only in the most superficial zone of the lesion and was separated by a basement membrane from the gliotic zone that marked the passage of the knife through the true central nervous tissue of the olfactory bulb.

There were no axons in the nerve fibre layer at the exact site of the incision at any of the post-operative times studied. Bundles of longitudinally oriented olfactory axons were only rarely observed immediately rostral or caudal to the lesion. At all times, however, these locations contained small bundles of transversely aligned axons, which were cut in cross section. By the 183rd post-operative day, an ectopic glomerulus was occasionally seen in the external plexiform layer immediately rostral to the incision (Fig. 7). Ectopic glomeruli are areas of neuropil that resemble normal olfactory glomeruli, but are situated in abnormal locations. They have been de-

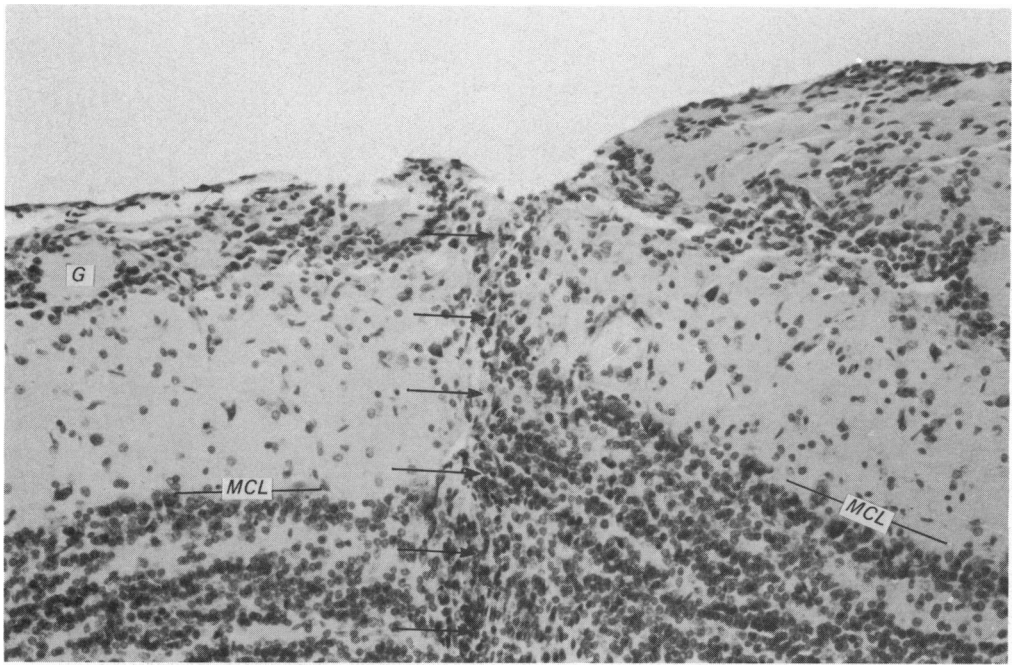


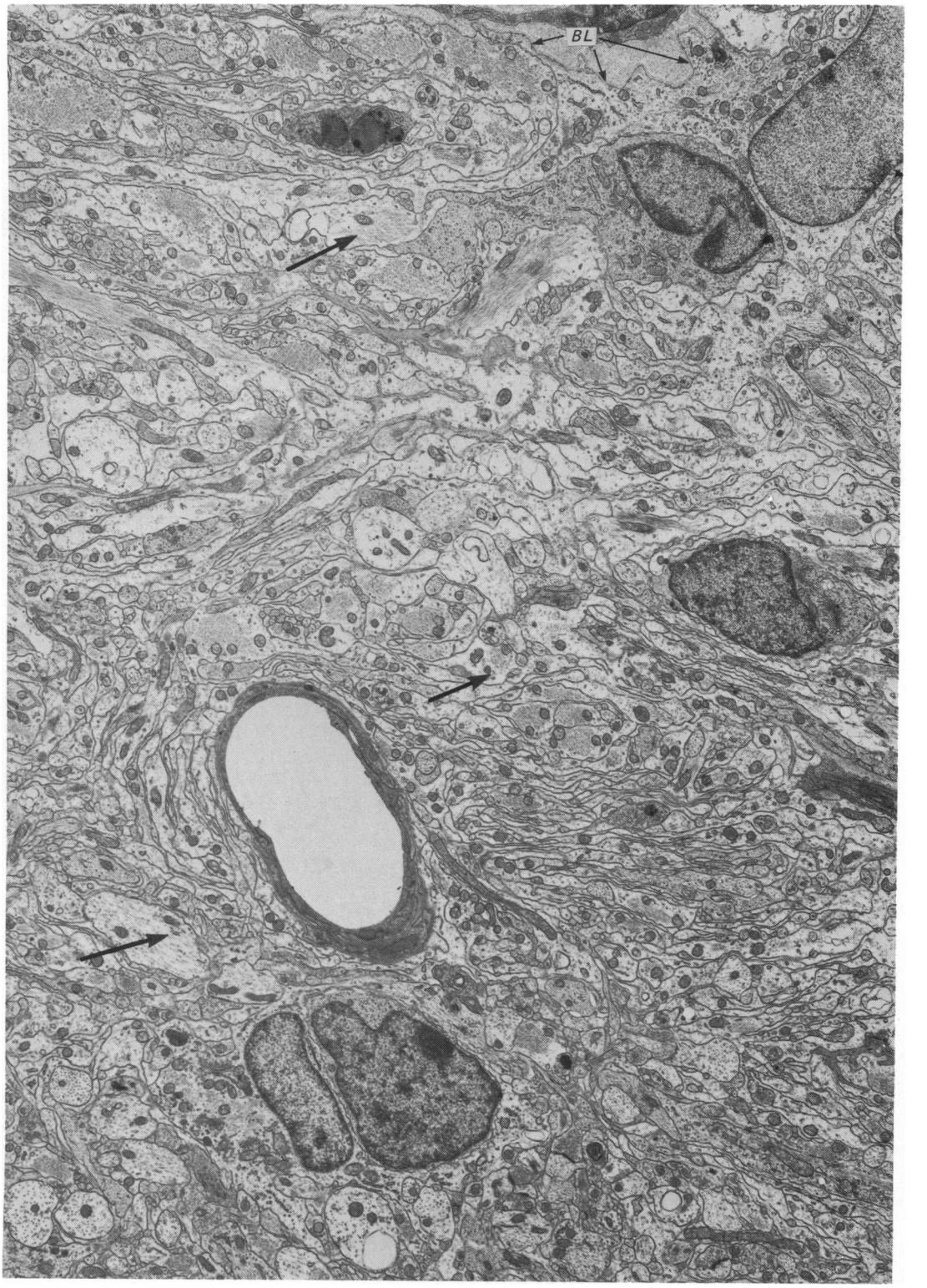
Fig. 5. Site of a transverse incision in the dorsal surface of the olfactory bulb, 14 days after operation. The line of the incision is indicated by arrows. Glomeruli (*G*) appear undistorted, but the mitral cell layer (*MCL*) and underlying granular layer have been displaced ventrally on the caudal (left hand) side of the lesion. Parasagittal paraffin section, stained with iron-haematoxylin and van Gieson. × 18.

scribed in the olfactory bulb and in other parts of the telencephalon following abnormally directed growth of axons from transected olfactory nerves (Graziadei & Samanen, 1980).

Caudal to incision in dorsal surface of olfactory bulb

As early as the second post-operative day, the neuroglial processes were more than normally large and numerous in the nerve fibre layer for a distance of 1·0–1·5 mm caudal to the lesion. Numerous astrocytic processes were also present in the underlying glomeruli. Small, dark, phagocytic cells, identified as reactive microglia, were present in both the nerve fibre layer and the glomeruli caudal to a transverse incision. These appearances persisted for three to four weeks. Swollen and degenerating axons were found in the nerve fibre layer caudal to the site of the incision from two to ten days after operation. During this time, there were also degenerating olfactory axonal terminals within the glomeruli caudal to the lesion (Fig. 8). The dendrites within these glomeruli were swollen on the second post-operative day, but were of normal size thereafter. Membranous and amorphous electron-dense inclusions were present in the dendrites, however, up to 183 days. The engulfment of degenerating boutons by dendrites was most commonly seen ten days after placement of the lesion. These

Fig. 6. Site of a transverse incision in the nerve fibre layer of the olfactory bulb, 40 days after operation. The most abundant elements are the cytoplasmic processes of hypertrophied astrocytes (some of the larger ones marked by arrows). The basal lamina of the external limiting glial membrane at the surface of the bulb is shown (*BL*) at the top of the picture. Electron micrograph. × 6050.


BL

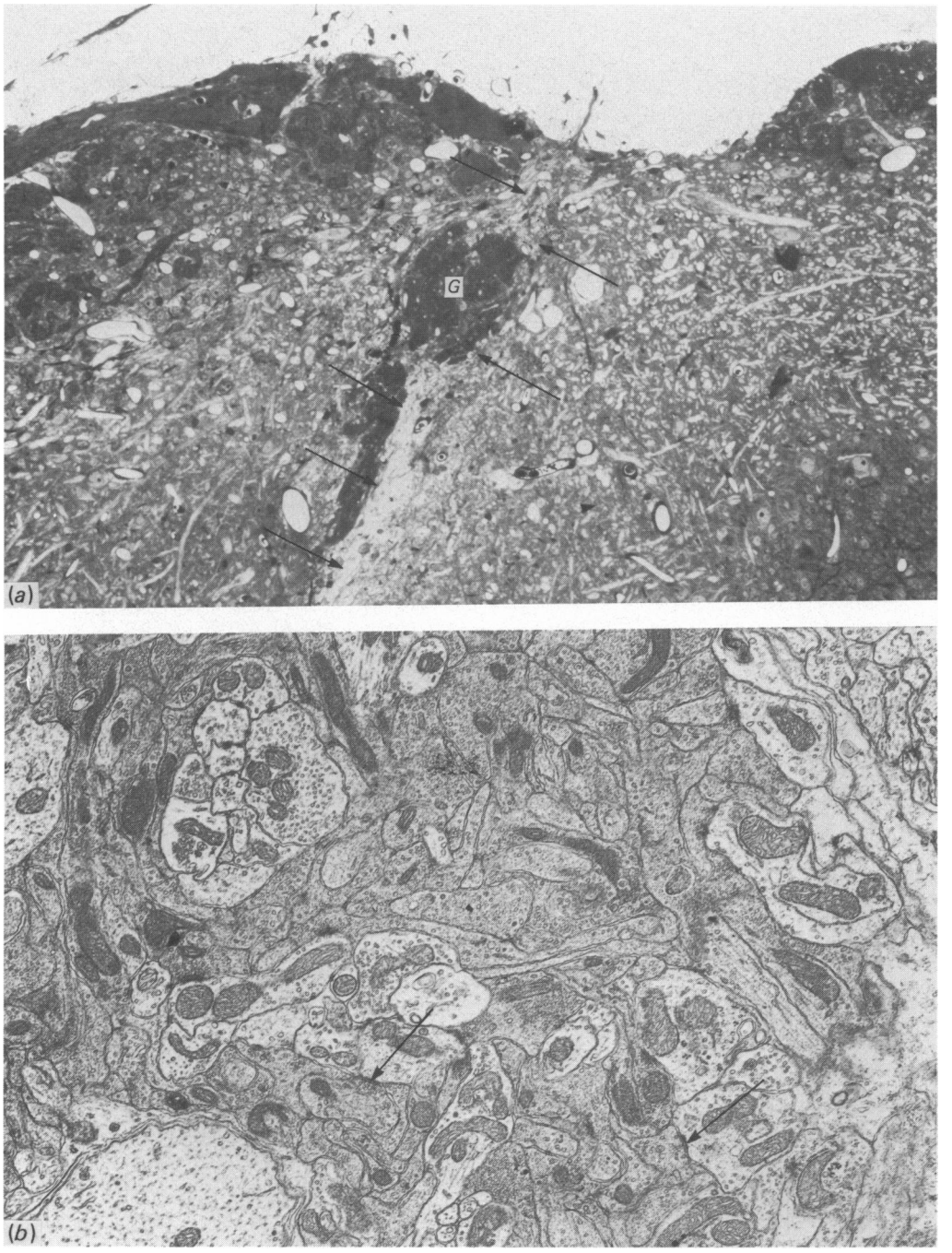


Fig. 7(*a–b*). Transverse incision in olfactory bulb, 183 days post-operatively. (*a*) An ectopic glomerulus (*G*) has developed in the external plexiform layer on the rostral (left hand) side of the lesion. Arrows mark the line of the incision. Plastic-embedded tissue; 1 μm section stained with alkaline toluidine blue. × 150. (*b*) Electron micrograph of the ectopic glomerulus. Olfactory axons are recognisable by virtue of their high electron density. Some axodendritic synapses are marked by arrows. × 11950.

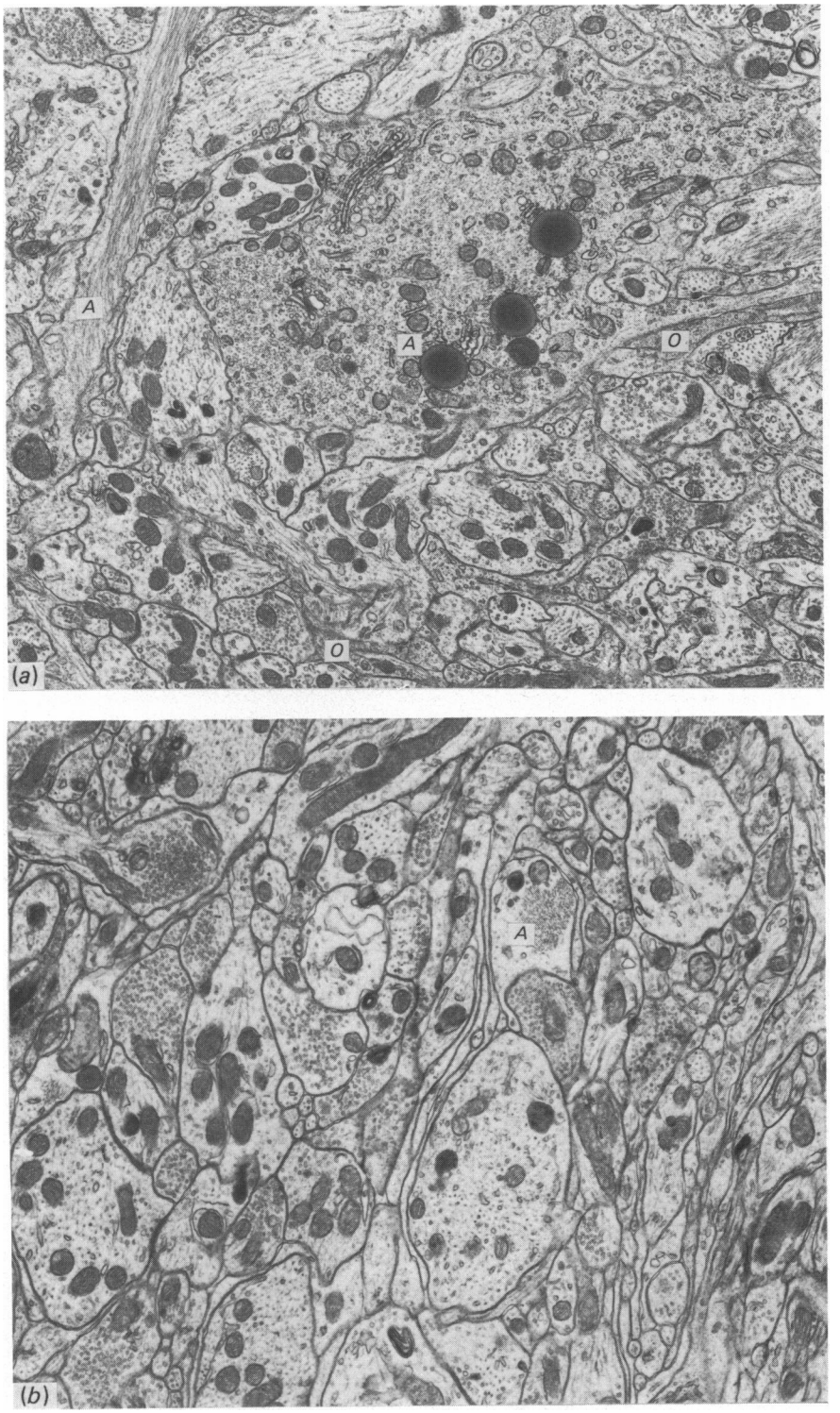


Fig. 8(*a–b*). Electron micrographs of glomeruli caudal to transverse incisions in the nerve fibre layer. (*a*) On the 4th post-operative day, the neuropil has become infiltrated by the cytoplasmic processes of astrocytes (*A*). Olfactory axons (*O*) show signs of degeneration and are much less numerous than in a normal glomerulus. × 9800. (*b*) On the 14th post-operative day, no olfactory axons remain. Most of the profiles in this field are of dendrites. An astrocyte process (*A*) is also present. × 15000.

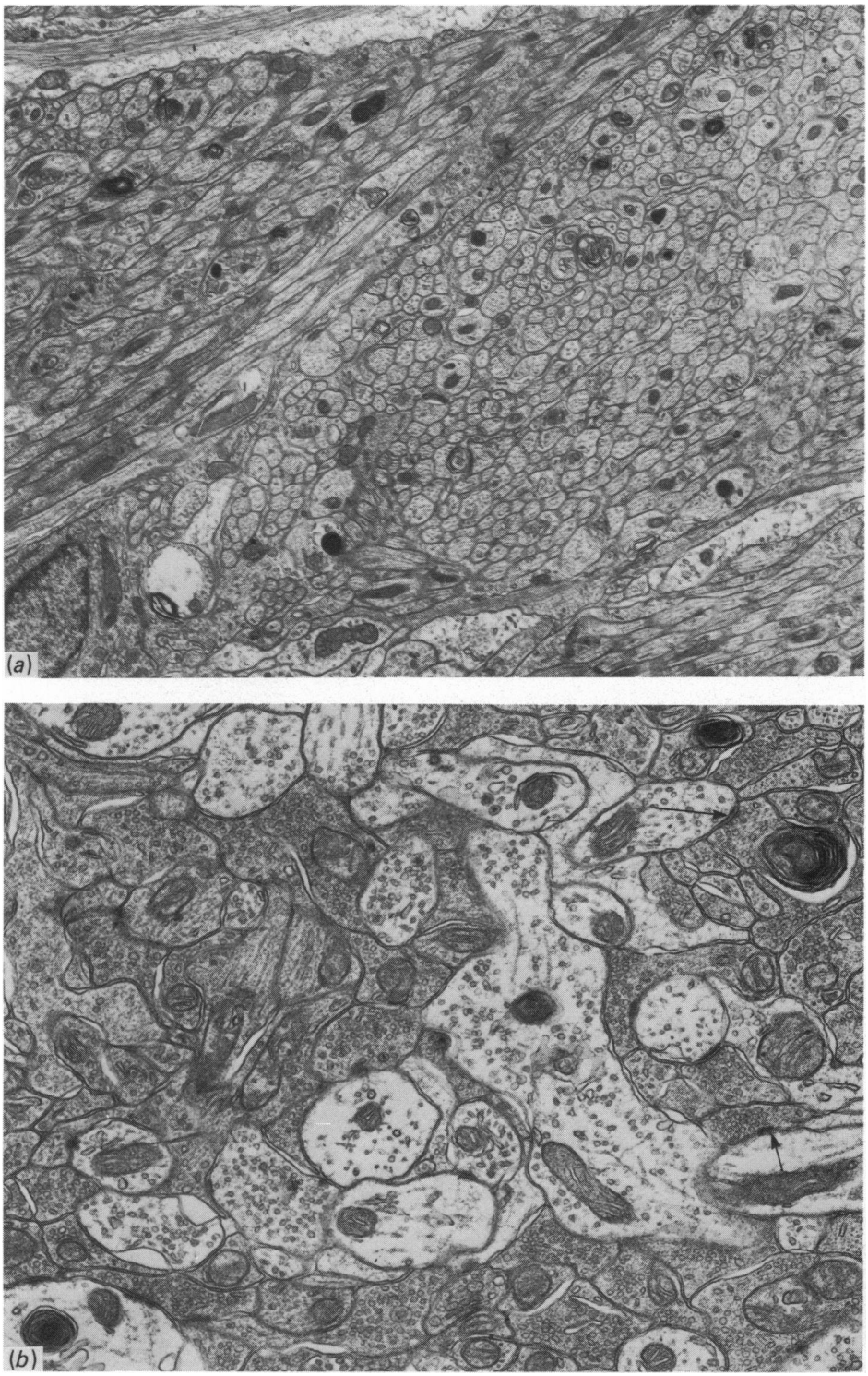


Fig. 9(*a–b*). Electron micrographs of the olfactory bulb caudal to the site of a transverse incision in the nerve fibre layer, on the 30th post-operative day. (*a*) Large bundles of primary olfactory axons in the nerve fibre layer. The fibres are cut both longitudinally and transversely. ×8800. (*b*) Glomerular neuropil containing numerous olfactory axons (dark) and axodendritic synapses (arrows). ×21000.

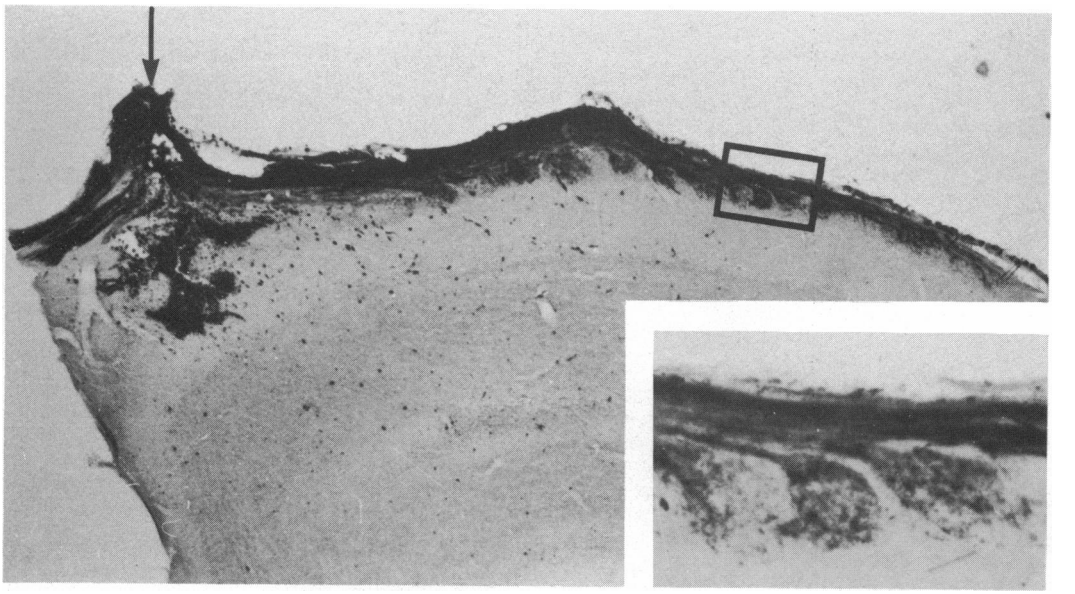


Fig. 10. In a normal rat, horseradish peroxidase was applied to a freshly made transverse incision in the dorsal surface of the olfactory bulb at the site shown by the arrow. After a survival of 24 hours, the enzyme had been transported caudally (to the right) in the axons of the nerve fibre layer and into the underlying glomeruli (enlarged in inset).

observations indicate that, as after transection of the olfactory nerves, the dendrites participate in the removal of degenerated presynaptic boutons.

By the 14th post-operative day, none of the glomeruli 1·0–1·5 mm caudal to the lesion contained any terminal boutons of primary afferent fibres (Fig. 8). These glomeruli had, therefore, been completely denervated. Bundles of olfactory axons of healthy appearance were present, however, in the nerve fibre layer above the denervated glomeruli, and in one animal there were a few intraglomerular axons, but no terminals. At 19 days, the glomeruli were still completely denervated in one animal. In other animals at 19 and 21 days after operation, some olfactory nerve fibres and terminals were present within the glomeruli, though large areas of the neuropil were still denervated. Thus, the glomeruli began to be re-innervated two to three weeks after the axons that formerly supplied them had been transected. At later times after placement of the lesion, large bundles of olfactory axons were present in the nerve fibre layer, and the glomeruli contained olfactory fibres and terminals whose numbers increased with time. Re-innervation was usually completed by the end of the fourth post-operative week (Fig. 9).

Anterograde transport of horseradish peroxidase

When horseradish peroxidase was applied to an incision in the surface of the olfactory bulb, the enzyme was detected 24 hours later in the nerve fibre layer and in glomeruli on the dorsal surface of the bulb for 1–3 mm posterior to the lesion (Fig. 10). This distribution corresponds with that of degenerating boutons seen after transection of the same axons in the same place. Thus, both methods showed that glomeruli on the dorsal aspect of the olfactory bulb received afferent fibres that passed rostrocaudally through the immediately rostral region of the nerve fibre layer.

The course of the re-innervation of olfactory glomeruli after transection of their

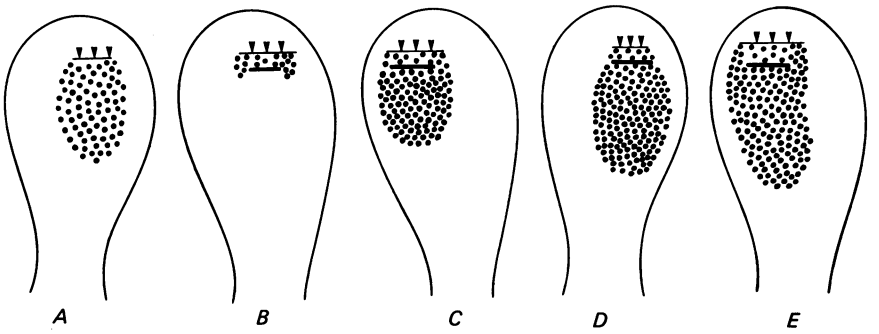


Fig. 11 (*A–E*). Drawings of the dorsal aspect of the right olfactory bulb, showing the distribution of glomeruli labelled by anterogradely transported horseradish peroxidase. The enzyme was applied at an incision along the line marked by arrowheads. The thicker line in (*B*), (*C*), (*D*) and (*E*) shows the site of an earlier transverse incision in the nerve fibre layer. Glomeruli caudal to (below) the thick line have been re-innervated. (*A*) Control animal (compare with Fig. 10). (*B* and *C*) Horseradish peroxidase was applied 14 days after the initial incision. (*D* and *E*) Horseradish peroxidase was applied 31 days after the initial incision.

afferent axons in the nerve fibre layer could be followed in rats in which horseradish peroxidase was applied to a second incision, slightly anterior to the original one, 14 or 31 days later. The results are plotted in Figure 11, where it can be seen that the glomeruli lateral and caudal to the first lesion were re-innervated after 14 days in one animal and after 31 days in two animals. In another animal, the glomeruli were not innervated after 14 days, even though labelled glomeruli were present medial and lateral to the incisions. In all four animals there was also dense labelling of the nerve fibre and glomerular layers in the zone between the two incisions. Labelled mitral cells were commonly observed in all the animals, a consequence, no doubt, of the fact that the second lesion always extended into the external plexiform layer of the olfactory bulb.

DISCUSSION

Identity of axons re-innervating glomeruli

It is fortunate that the terminal parts of the primary olfactory axons are easily recognisable in electron micrographs by virtue of their electron-dense cytoplasm. No other cellular processes in the olfactory bulb resemble them (Shepherd, 1972, 1977). Consequently, it can be stated with confidence that following denervation, as the result of a lesion in either the peripheral or the central nervous system, the glomeruli were re-innervated only by the axons of the olfactory receptor neurons.

Absence of axonal sprouting

It is noteworthy that at no time were the denervated dendrites in the glomeruli contacted by any other neuronal processes. There is evidently no sprouting of intact axons within the denervated olfactory bulb. Such sprouting, also known as 'plasticity', occurs following de-afferentation of several regions of the nervous system including the spinal grey matter (Liu & Chambers, 1958), the septal area (Raisman, 1969) and the hippocampal formation (Cotman & Nadler, 1978). In these regions, the postsynaptic sites vacated by degenerated boutons become re-occupied by newly formed branches of neighbouring axons. The olfactory bulb resembles the trigeminal nuclear complex (Kerr, 1972) and the cerebellar cortex (Anderson, 1981), regions in which little or no axonal sprouting follows the transection of afferent fibres. In the

brains of very young animals, axonal sprouting following injury can lead to conspicuous, persistent abnormalities of neuronal connectivity (Lund, 1978). In adults, however, Goldberger & Murray (1978) and Cotman, Nieto-Sampedro & Harris (1981) have pointed out that sprouting axons never grow outside the bounds of their normal fields of projection. This rule evidently applies to the olfactory bulb, in which no axons other than those from the olfactory nerve are normally present within the glomeruli.

The primary olfactory axons themselves belong to neurons that are, perhaps, never fully mature. They might, therefore, be expected to sprout outside the territory of their glomeruli if the neighbouring external plexiform layer of the olfactory bulb were partially de-afferented. This possibility could be tested by examining the external plexiform layer at various intervals of time after destruction of its afferent neurons, which are located mainly in the anterior olfactory nucleus and the nucleus of the diagonal band (Price & Powell, 1970*a*, *b*).

Absence of true axonal regeneration

The length of time required for re-innervation of glomeruli on the dorsal surface of the olfactory bulb was approximately the same when the afferent axons had been transected at the level of the cribriform plate or within the nerve fibre layer of the bulb. It is, therefore, most unlikely that the severed axons regenerated by growing distally from their proximal stumps in the same way as those of an ordinary peripheral nerve. Had such been the case, re-innervation would have occurred much more rapidly after cutting the axons in the bulb than after cutting the olfactory nerves. Our results indicate that the primary olfactory axons do not regenerate from the site of axotomy, whether this be within the territory of the central or the peripheral nervous system. Further evidence against the occurrence of true axonal regeneration (as defined by Kiernan, 1979) in the primary olfactory pathway derives from the degeneration and disappearance of the neurons in the olfactory epithelium following transection of their axons (Graziadei & DeHan, 1973; Harding *et al.* 1977). The retrograde degeneration occurs sooner after transection at the cribriform plate than after transection in the nerve fibre layer of the bulb. This difference is probably not due to the distances of lesions of the two types from the neuronal somata. Possible reasons for the discrepancy have been discussed by Doucette *et al.* (1983).

We conclude that the axons re-innervating the glomeruli were those of new neurons that had differentiated in the nasal epithelium. The elongating axons of such neurons had to cover the same distances irrespective of the sites at which their predecessors had been transected, so the time required for re-innervation of the glomeruli was the same for both types of lesion.

Origin and course of re-innervating axons

After transection of most of the olfactory nerves at the cribriform plate, the axons of the new receptor neurons must grow through the site of the lesion, there being no other route by which they could reach the olfactory bulb. The reunited olfactory nerves are clearly visible in paraffin sections of decalcified specimens (Doucette *et al.* 1983), but we have been unable to follow the courses of the individual axons because, despite many attempts, they cannot be adequately stained by silver methods. This difficulty is probably due to the fact that the diameters of the primary olfactory axons are close to the limit of resolution of the light microscope while the individual fibres are separated from one another by distances far too small to be optically resolvable.

Having grown across the site of the lesion, the new axons must then traverse the

junction between peripheral and central nervous tissue. Their ensheathing cells change from the Schwann cells of the olfactory nerve to the central neuroglia of the most superficial layer of the olfactory bulb. This junctional zone has not yet been studied with the electron microscope, but it may differ from the equivalent one in the dorsal spinal roots which has been described in detail by Berthold & Carlstedt (1977). It may also be significant that the neuroglial cells ensheathing the axons in the nerve fibre layer of the olfactory bulb have several distinctive ultrastructural features (Doucette, unpublished observations). It is not yet known whether these neuroglial cells have the special property of permitting the entry of growing axons into the olfactory bulb. The failure of regenerating axons to grow from a dorsal root into the spinal cord (Paskind, 1936; Kimmel & Moyer, 1947; Taub, Harger, Grier & Hodos, 1980) is sometimes attributed to a barrier formed by astrocytes (Berthold & Carlstedt, 1977; Stensaas, Burgess & Horch, 1979), though other explanations have also been suggested (Berry, 1979; Kiernan, 1979). Finally, the growing axons must enter the denervated glomeruli, where they are no longer ensheathed by neuroglial cells, and synapse with the dendrites of the mitral, tufted and periglomerular cells. The full complement of presynaptic terminals is restored gradually, between the third and sixth post-operative weeks. The slow progress of the re-innervation is probably due partly to the different lengths of the olfactory nerve fibres supplying each glomerulus (Doucette *et al.* 1983) and partly to the time taken up by the growth of terminal branches from the intraglomerular portions of the axons.

The course followed by newly growing axons after transection of their predecessors on the dorsal surface of the olfactory bulb is evidently similar to that discussed above for the events that follow transection at the cribriform plate. However, both electron microscopy and anterograde tracing with horseradish peroxidase indicate that none of the new axons grow through the site of the lesion in the nerve fibre layer of the bulb. The axons must, therefore, reach the glomeruli behind the lesion by some other route. Horseradish peroxidase applied rostral to the original lesion, after re-innervation had taken place, was distributed to the glomeruli that had been denervated. Thus, the axons of the new neurons followed the same course as their predecessors, almost up to the site of transection. In order to reach the glomeruli on the other side of that site, the axons could have been diverted either beneath the lesion, through the external plexiform or granule cell layer, or around the lesion, in the nerve fibre layer. The occasional development of an ectopic glomerulus within the external plexiform layer immediately rostral to the lesion indicates that some axons were deflected into the deeper layers of the bulb, formed ectopic synapses there, and grew no further. We saw no evidence of any olfactory axons in passage within the deeper parts of the bulb at and around the site of the transverse incision. On the other hand, the distribution of glomeruli labelled with horseradish peroxidase (see Fig. 11), and the preponderance of transversely oriented axons in the nerve fibre layer immediately rostral and caudal to the lesion, strongly suggest that the re-innervating axons grew around the ends of the incision, within the most superficial layer of the olfactory bulb.

The growth of axons through uninjured central nervous tissue near a wound may not be unique to the olfactory system. Foerster (1980, 1982) has presented evidence for the regenerative growth of axons around lesions in the adult rat's brain. Moulton-Barrett & Berry (1980) and McConnell & Berry (1982) have demonstrated extensive, though misdirected, axonal growth in the retina after transection of the axons of the ganglion cells in the adult mouse. Their observations were facilitated by examining

the retina as a whole-mount. In most other parts of the brain and spinal cord, the necessity for sectioning the tissue may prevent the observation of axons growing tortuously in several directions.

SUMMARY

The re-innervation of the olfactory bulb has been studied in rats in which the primary afferent axons were transected either in the peripheral nervous system, on the intracranial side of the cribriform plate, or in the central nervous system, in the nerve fibre layer of the bulb. Both procedures resulted in denervation of glomeruli on the dorsal surface of the olfactory bulb. Re-innervation of these glomeruli was first seen approximately three weeks after operation and was largely completed by the sixth week, irrespective of the site of the lesion. The similarity of the timing of re-innervation following the two procedures indicates that the cut fibres did not re-generate from their sites of transection. It is much more probable that the re-innervating axons were those of neurons newly generated in the olfactory epithelium. This view is supported by the results of other investigations, in which retrograde degeneration and subsequent replacement of the neurons have been found to follow transection of the olfactory nerves.

After transection of the olfactory nerves, the new axons entering the bulb grew through the site of the lesion, across the interface between peripheral and central nervous tissue, through the nerve fibre layer and into the glomeruli. Thus, they followed the same course as normally growing primary olfactory axons. After the afferent fibres had been cut within the olfactory bulb, the site of transection was transformed into a scar composed largely of astrocytes. No olfactory axons grew through the scar and none passed beneath it in the deeper layers of the bulb. However, by tracing the anterograde axonal transport of horseradish peroxidase, it has been shown that axons immediately rostral to the lesion terminated in the re-innervated glomeruli. These denervated glomeruli were, therefore, probably re-innervated by axons that grew through the intact central nervous tissue of the nerve fibre layer on either side of the lesion.

This work was supported by grants from the Multiple Sclerosis Society of Canada to J.A.K. and the Medical Research Council to B.A.F. We are grateful to Mrs Sharon Griffin and Mrs Judy Sholdice for technical assistance.

REFERENCES

ANDERSON, W. A. (1981). The effects of deafferentation of the cerebellar cortex in the rat. Ph.D. thesis, University of Western Ontario, Canada.

ANDERSON, W. A. & FLUMERFELT, B. A. (1980). A light and electron microscopic study of the effects of 3-acetylpyridine intoxication on the inferior olivary complex and cerebellar cortex. *Journal of Comparative Neurology* **190**, 157–174.

BERRY, M. (1979). Regeneration in the central nervous system. In *Recent Advances in Neuropathology*, vol. 1 (ed. J. Cavanagh & W. T. Smith), pp. 67–111. Edinburgh & London: Churchill Livingstone.

BERTHOLD, C. H. & CARLSTEDT, T. (1977). Observations on the morphology at the transition between the peripheral and the central nervous system in the cat: II. General organization of the transitional region in S1 dorsal rootlets. *Acta physiologica scandinavica, Suppl.* **446**, 23–42.

COTMAN, C. W. & NADLER, J. V. (1978). Reactive synaptogenesis in the hippocampus. In *Neuronal Plasticity* (ed. C. W. Cotman), pp. 227–271. New York: Raven Press.

COTMAN, C. W., NIETO-SAMPEDRO, M. & HARRIS, E. W. (1981). Synapse replacement in the nervous system of adult vertebrates. *Physiological Reviews* **61**, 684–784.

DOUCETTE, J. R. (1981). Regeneration and plasticity in the olfactory system of the rat. Ph.D. thesis, University of Western Ontario, Canada.

DOUCETTE, J. R., KIERNAN, J. A. & FLUMERFELT, B. A. (1983). Two different patterns of retrograde degeneration in the olfactory epithelium following transection of primary olfactory axons. *Journal of Anatomy* **136**, 673–689.

FOERSTER, A. P. (1980). Axonal regeneration in the adult rat brain. *Proceedings of the Canadian Federation of Biological Societies* **23**, 16.

FOERSTER, A. P. (1982). Spontaneous regeneration of cut axons in adult rat brain. *Journal of Comparative Neurology* **210**, 335–356.

FUJITA, S. & KITAMURA, T. (1976). Origin of brain macrophages and the nature of the microglia. In *Progress in Neuropathology*, vol. 3 (ed. H. M. Zimmerman), pp. 1–50. New York: Grune & Stratton.

GOLDBERGER, M. E. & MURRAY, M. (1978). Recovery of movement and axonal sprouting may obey some of the same laws. In *Neuronal Plasticity* (ed. C. W. Cotman), pp. 73–96. New York: Raven Press.

GRAZIADEI, P. P. C. & DEHAN, R. S. (1973). Neuronal regeneration in frog olfactory system. *Journal of Cell Biology* **59**, 525–530.

GRAZIADEI, P. P. C. & MONTI GRAZIADEI, G. A. (1976). Synaptic changes and reinnervation of olfactory glomeruli of mouse, following section of fila-olfactoria. *Anatomical Record* **184**, 416.

GRAZIADEI, P. P. C. & MONTI GRAZIADEI, G. A. (1978*a*). Continuous nerve cell renewal in the olfactory system. In *Handbook of Sensory Physiology*, vol. 9 (ed. M. Jacobson), pp. 55–82. Berlin: Springer-Verlag.

GRAZIADEI, P. P. C. & MONTI GRAZIADEI, G. A. (1978*b*). The olfactory system: a model for the study of neurogenesis and axon regeneration in mammals. In *Neuronal Plasticity* (ed. C. W. Cotman), pp. 113–153. New York: Raven Press.

GRAZIADEI, P. P. C. & MONTI GRAZIADEI, G. A. (1979). Neurogenesis and neuron regeneration in the olfactory system of mammals. I. Morphological aspects of differentiation and structural organization of the olfactory sensory neurons. *Journal of Neurocytology* **8**, 1–18.

GRAZIADEI, P. P. C. & MONTI GRAZIADEI, G. A. (1980). Neurogenesis and neuron regeneration in the olfactory system of mammals. III. Deafferentation and reinnervation of the olfactory bulb following section of the fila olfactoria in rat. *Journal of Neurocytology* **9**, 145–162.

GRAZIADEI, P. P. C. & SAMANEN, D. W. (1980). Ectopic glomerular structures in the olfactory bulb of neonatal and adult mice. *Brain Research* **187**, 467–472.

HARDING, J., GRAZIADEI, P. P. C., MONTI GRAZIADEI, G. A. & MARGOLIS, F. L. (1977). Denervation in the primary olfactory pathway of mice. IV. Biochemical and morphological evidence for neuronal replacement following nerve section. *Brain Research* **132**, 11–28.

KERR, F. W. L. (1972). The potential of cervical primary afferents to sprout into the spinal nucleus of V following long term trigeminal denervation. *Brain Research* **42**, 547–560.

KIERNAN, J. A. (1979). Hypotheses concerned with axonal regeneration in the mammalian nervous system. *Biological Reviews* **54**, 155–197.

KIMMEL, D. L. & MOYER, E. K. (1947). Dorsal roots following anastomosis of the central stumps. *Journal of Comparative Neurology* **87**, 289–319.

LIU, C. N. & CHAMBERS, W. W. (1958). Intraspinal sprouting of dorsal root axons. *Archives of Neurology and Psychiatry* **79**, 46–61.

LUND, R. D. (1978). *Development of Plasticity of the Brain*. New York: Oxford University Press.

MCCONNELL, P. & BERRY, M. (1982). Regeneration of axons in mouse retina after injury. In *Bibliotheca Anatomica* 3. Basel: Karger.

MESULAM, M. M. (1978). Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. *Journal of Histochemistry and Cytochemistry* **26**, 106–117.

MESULAM, M. M., HEGARTY, E., BARBAS, H., CARSON, K. A., GOWER, E. C., KNAPP, A. G., MOSS, M. B. & MUFSON, E. J. (1980). Additional factors influencing sensitivity in the tetramethyl benzidine method for horseradish neurohistochemistry. *Journal of Histochemistry and Cytochemistry* **28**, 1255–1259.

MONTI GRAZIADEI, G. A. & GRAZIADEI, P. P. C. (1979). Neurogenesis and neuron regeneration in the olfactory system of mammals. II. Degeneration and reconstitution of the olfactory sensory neurons after axotomy. *Journal of Neurocytology* **8**, 197–213.

MONTI GRAZIADEI, G. A., KAPLAN, M. S., BERNSTEIN, J. J. & GRAZIADEI, P. P. C. (1980). Reinnervation of the olfactory bulb after section of the olfactory nerve in monkey (*Saimiri sciureus*). *Brain Research* **189**, 343–354.

MOULTON-BARRETT, R. & BERRY, M. (1980). Preliminary quantitative study of abortive regeneration of ganglion cell axons in the retina of the adult mouse. *Journal of Anatomy* **131**, 768–769.

OEHMICHEN, M. (1978). *Mononuclear Phagocytes in the Central Nervous System.* (trans. M. M. Clarkson). Berlin: Springer-Verlag.

OLEY, N., DEHAN, R. S., TUCKER, D., SMITH, J. C. & GRAZIADEI, P. P. C. (1975). Recovery of structure and function following transection of the primary olfactory nerves in pigeons. *Journal of Comparative and Physiological Psychology* **88**, 477–495.

PALAY, S. L. & CHAN-PALAY, V. (1974). *Cerebellar Cortex: Cytology and Organization.* Berlin: Springer-Verlag.

PASKIND, H. A. (1936). Regeneration in the posterior root fibers in the cat. *Archives of Neurology and Psychiatry* **36**, 1077–1084.

PINCHING, A. J. & POWELL, T. P. S. (1971). The neuropil of the glomeruli of the olfactory bulb. *Journal of Cell Science* **9**, 347–377.

PRICE, J. L. & POWELL, T. P. S. (1970*a*). An electron microscopic study of the termination of the afferent fibres to the olfactory bulb from the cerebral hemisphere. *Journal of Cell Science* **7**, 157–187.

PRICE, J. L. & POWELL, T. P. S. (1970*b*). An experimental study of the origin and the course of the centrifugal fibres to the olfactory bulb in the rat. *Journal of Anatomy* **107**, 215–237.

RAISMAN, G. (1969). Neuronal plasticity in the septal nuclei of the adult rat. *Brain Research* **14**, 25–48.

SHEPHERD, G. M. (1972). Synaptic organization of the mammalian olfactory bulb. *Physiological Reviews* **52**, 864–917.

SHEPHERD, G. M. (1977). The olfactory bulb: a simple system in the mammalian brain. In *Handbook of Physiology, Section* 1: *The Nervous System*, vol. 1 (ed. J. M. Brookhart & V. B. Mountcastle), pp. 945–968. Bethesda, Maryland: American Physiological Society.

SIMMONS, P. A., RAFOLS, J. A. & GETCHELL, T. V. (1981). Ultrastructural changes in olfactory receptor neurons following olfactory nerve section. *Journal of Comparative Neurology* **45**, 529–549.

STENSAAS, L. J., BURGESS, P. R. & HORCH, K. W. (1979). Regenerating dorsal root axons are blocked by spinal cord astrocytes. *Society for Neuroscience Abstracts* **5**, 684.

TAKAGI, S. F. (1971). Degeneration and regeneration of the olfactory epithelium. In *Handbook of Sensory Physiology*, vol. IV (ed. L. M. Beidler), ch. 4, pp. 75–94. Berlin: Springer-Verlag.

TAUB, E., HARGER, M., GRIER, C. H. & HODOS, W. (1980). Some anatomical observations following chronic dorsal rhizotomy in monkeys. *Neuroscience* **5**, 389–401.

VALENSTEIN, E. S. (1961). A note on anesthetizing rats and guinea pigs. *Journal of the Experimental Analysis of Behavior* **4**, 6.

VENABLE, J. H. & COGGESHALL, R. (1965). A simplified lead citrate stain for use in electron microscopy. *Journal of Cell Biology* **25**, 407.

WALBERG, F. (1963). Role of normal dendrites in removal of degenerating terminal boutons. *Experimental Neurology* **8**, 112–124.

WILLEY, T. J. (1973). The ultrastructure of the cat olfactory bulb. *Journal of Comparative Neurology* **152**, 211–232.

WISNIEWSKI, H. M., GHETTI, B. & HOROUPIAN, D. S. (1972). The fate of synaptic membranes of degenerating optic nerve terminals, and their role in the mechanism of trans-synaptic changes. *Journal of Neurocytology* **1**, 297–310.