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Two different patterns of retrograde degeneration in the olfactory epithelium following transection of primary olfactory axons

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INTRODUCTION

The olfactory epithelium is the only region of the nervous system in adult mammals in which neurons die and are regularly replaced. Basal cells divide and differentiate into receptor cells, and their axons then grow to the olfactory bulb where they form synapses within glomeruli. By labelling dividing cells with [^{3}H]thymidine it has been shown that in the rat and the mouse each neuron in the olfactory epithelium lives for 30 to 40 days (Graziadei & Monti Graziadei, 1978*a*, 1979).

It is widely held that transection of the olfactory nerves is quickly followed by death of the olfactory receptor cells. New receptor cells are then formed and their axons grow through the site of the lesion and re-innervate the glomeruli of the olfactory bulb (Graziadei & Monti Graziadei, 1978*b*; Monti Graziadei & Graziadei, 1979; Graziadei & Okano, 1979; Graziadei, Kaplan, Monti Graziadei & Bernstein, 1980; Simmons, Rafols & Getchell, 1981). We have found, however, that axonal injury does not always result in prompt death of the olfactory receptor cells (Doucette, 1981). We now show that retrograde changes in the olfactory epithelium vary with the site of axonal transection. Some possible mechanisms whereby the olfactory bulb influences the life spans of neurons in the nasal epithelium are suggested on the basis of these observations.

MATERIALS AND METHODS

The animals used were male, albino Wistar rats weighing 200–425 g. For operative procedures, including perfusion of fixative and killing by decapitation, the animals were anaesthetised with an intraperitoneally injected combination of pentobarbitone and chloral hydrate (Valenstein, 1961).

This communication is concerned with the olfactory epithelia of the experimental animals. The changes in the olfactory bulbs of the same rats form part of another paper (Doucette, Kiernan & Flumerfelt, 1983).

Normal material

The heads of nine rats were fixed by immersion in Carnoy's fluid, Helly's fluid, Bouin's fluid or phosphate-buffered 2·5% formaldehyde (see Kiernan (1981) for compositions and methods of use of these mixtures). Specimens containing the nasal

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apparatus and the rostral end of the brain of each animal were decalcified in formic acid–sodium formate buffer, pH 2 (Clark, 1954) and then double embedded in celloidin and paraffin wax by the method of Pfuhl, as described by Gabe (1976). Sections cut in the transverse, horizontal and sagittal planes were stained by the iron-haematoxylin and van Gieson method (Culling, 1974).

Eleven rats were perfused with a buffered glutaraldehyde–formaldehyde solution (Palay & Chan-Palay, 1974) and fixation was continued by immersion for 18 hours at 4 °C. Specimens no more than 2 mm in any dimension, taken from the olfactory mucosa and from the dorsal surfaces of the olfactory bulbs, were post-fixed in 2 % osmium tetroxide in 0·12 M phosphate buffer containing 7 % dextrose. These specimens were then contrast-stained *en bloc* with uranyl acetate, dehydrated, and embedded in an Epon–Araldite mixture (Anderson & Flumerfelt, 1980). Sections 0·5 or 1 μm thick were stained with 1 % toluidine blue in 1 % borax for optical microscopy. Ultrathin sections were mounted on copper grids and stained with lead citrate (Venable & Coggeshall, 1965) for electron microscopy.

Transection of olfactory nerves at cribriform plate

In 35 rats the right olfactory bulb was exposed by drilling through the overlying frontal bone. The olfactory nerve filaments were severed by passing the blade of a cataract knife through the cleft between the bulb and the underlying cribriform plate of the ethmoid bone. Postoperative intervals ranged from 3 to 84 days. The heads of 21 rats were then fixed by immersion for 3 days in buffered formaldehyde, decalcified, trimmed and embedded as described above. Sections were cut at 5, 10, and 15 μm in the transverse or the horizontal plane and stained with iron-haematoxylin and van Gieson's stain. The sections were used to assess the extent of the lesion and the changes in the olfactory epithelium.

For subsequent electron microscopy, 14 rats were perfused with glutaraldehyde–formaldehyde solution. Specimens were embedded in plastic and sectioned as described for the normal material. The specimens were all carefully oriented and numbered, to permit later identification of their sites of origin in the nose or olfactory bulb.

Removal of glomeruli from olfactory bulb

In 4 rats, a small lesion was made by aspirating tissue from the dorsal surface of the olfactory bulb through a hollow needle. The site of this injury, which removed a piece of the olfactory nerve fibre layer with the underlying glomeruli, was immediately behind that at which transverse incisions, to be described in the next paragraph, were made in other animals. After 7 days, the rats were killed. Their olfactory bulbs were removed, fixed in Carnoy's fluid, embedded in wax and sectioned serially at 15 μm in the sagittal plane. The remaining anterior portions of the heads were fixed in Helly's fluid, decalcified, trimmed, double embedded and sectioned in the transverse plane for examination of the olfactory epithelium. The sections were stained with iron-haematoxylin and van Gieson's stain. These four animals were studied in order to check earlier reports (Clark & Warwick, 1946; Clark, 1951) of retrograde degeneration in the olfactory epithelium following removal of the terminal parts of some of the primary axons together with their postsynaptic structures.

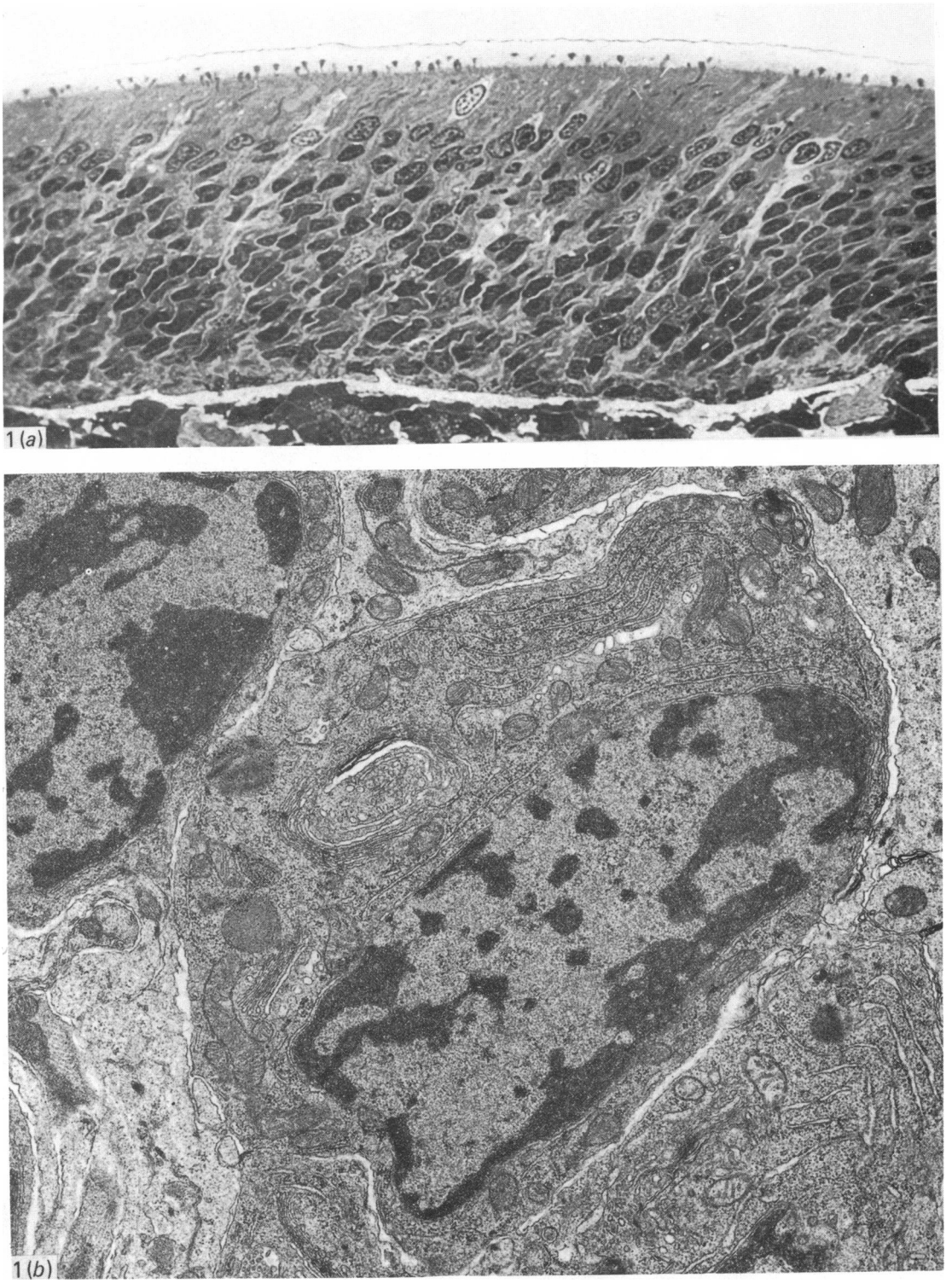


Fig. 1. Normal olfactory epithelium. (*a*) Plastic embedded semi-thin section, stained with toluidine blue. × 400. (*b*) Nucleus and perikaryon of a neuron. Electron micrograph. × 16750.

Transection of olfactory axons on dorsal surface of bulb

In 40 rats the dorsal surface of the right olfactory bulb was exposed and an incision approximately 0·5 mm deep was made in the transverse plane, across the dorsal surface of the bulb. This transverse incision, which passed through the nerve fibre and glomerular layers, was located 2 mm caudal to the rostral pole of the olfactory bulb. Animals were allowed to survive for 2 to 183 days after this operation. The olfactory bulbs of 13 of these rats were fixed in Carnoy's fluid and sectioned longitudinally in paraffin at 15 μm, to check the positions of the lesions. The noses of these animals were fixed in either buffered formaldehyde or Helly's fluid, decalcified and embedded in paraffin. Transverse sections 5, 10 and 15 μm thick were cut, for examination of the olfactory epithelium. All the paraffin sections were stained with iron-haematoxylin and van Gieson's stain. The remaining 27 rats were perfused with glutaraldehyde–formaldehyde solution. Specimens of olfactory epithelium and olfactory bulb were processed and embedded in plastic as described above. Sections were prepared for light and electron microscopy, as described for the normal material.

RESULTS

Normal olfactory epithelium

The olfactory epithelium was pseudostratified and consisted of supporting, receptor and basal cells. The elongated nuclei of the basal cells lay just above the basement membrane of the epithelium; those of the supporting cells formed a single layer near the luminal surface. Several layers of receptor cell nuclei were situated between the nuclei of the supporting and basal cells. Dendrites of the olfactory receptor cells reached the luminal surface where they terminated as olfactory vesicles (Fig. 1*a*).

At the ultrastructural level (Fig. 1*b*) the olfactory receptor cells were easily identified by virtue of their electron-dense cytoplasmic matrix. This density was also a useful identifying feature for the axons and terminal boutons of the receptor neurons (see also Pinching & Powell, 1971*a*, *b*; Willey, 1973; Shepherd, 1977). Each receptor cell contained a prominent Golgi apparatus, well developed rough endoplasmic reticulum, and numerous free ribosomes and mitochondria. Vacuolation of the Golgi apparatus and the presence of multivesicular and dense bodies were noted in many of the receptor cells located in the apical portion of the epithelium. Bundles of axons ensheathed by Schwann cells were observed in the lamina propria. The occurrence of many unmyelinated fibres within one mesaxon was a useful criterion for the identification of olfactory axons (DeLorenzo, 1957; Frisch, 1967). Ordinary nerves, in which the unmyelinated fibres had individual mesaxons, were also seen coursing through the lamina propria; they were presumed to contain both general sensory axons derived from the trigeminal nerve and postganglionic autonomic fibres.

Occasional degenerating cells were observed in the olfactory epithelia of all the normal animals (Fig. 2). Cells with pyknotic nuclei were found both at the base of the epithelium and near the luminal surface. The cytoplasm was electron-dense and contained no recognisable organelles. Near the luminal surface there were also cells with electron-lucent cytoplasm that contained few organelles but many membranous whorls. The nuclear membranes of these cells were still intact and the nuclei contained large clumps of chromatin. Degenerating cells of both types were embraced

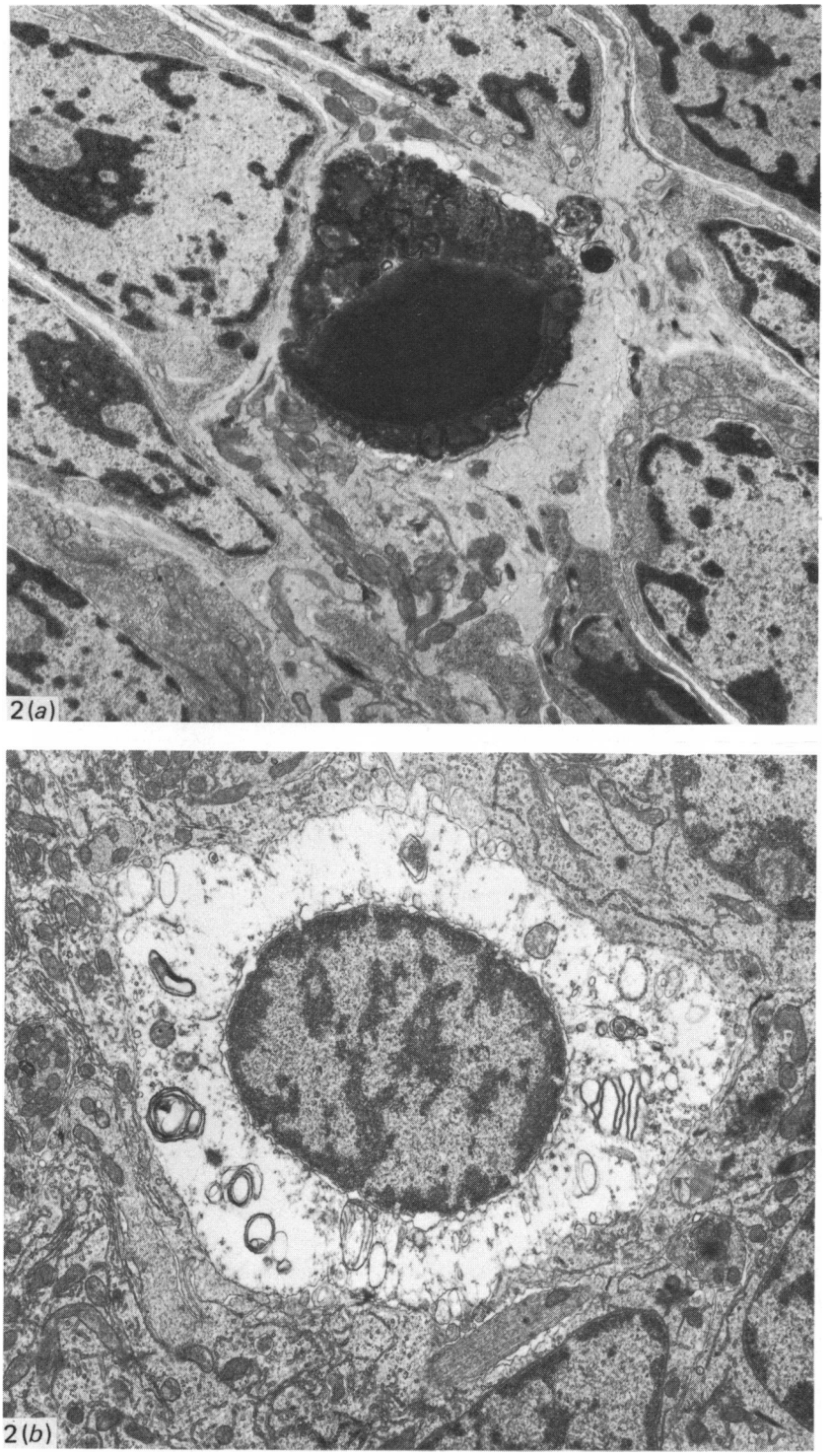


Fig. 2. Electron micrographs showing two degenerating neurons in normal olfactory epithelium. (*a*) With electron-dense cytoplasm. × 8890. (*b*) With electron-lucent cytoplasm. × 9330.

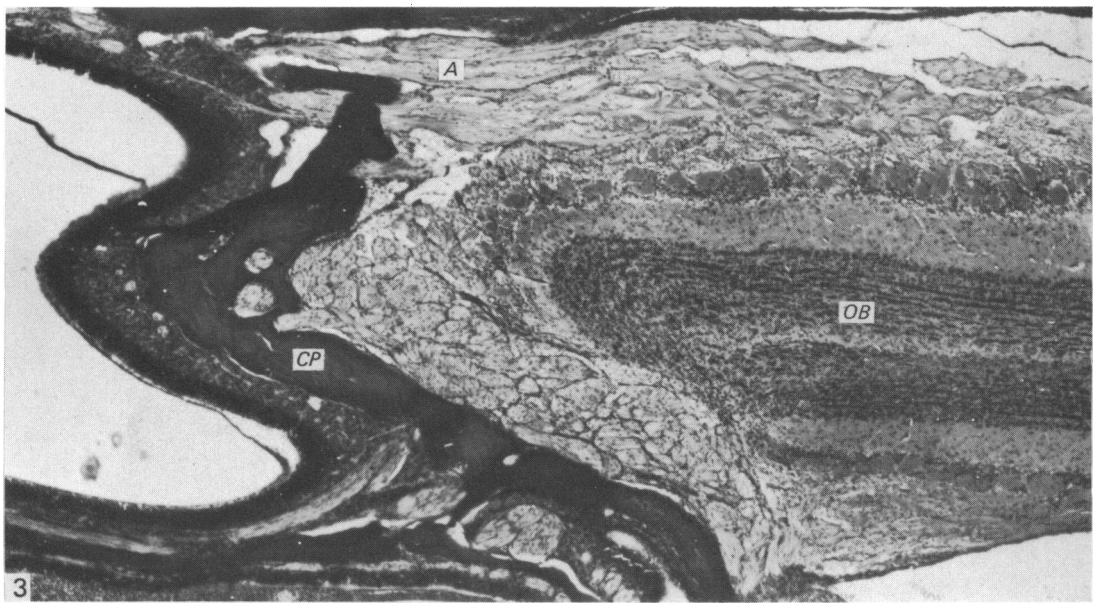


Fig. 3. Horizontal section through the head of a rat, showing the nasal cavity and the olfactory bulb (*OB*). The olfactory nerves had been transected, 12 days previously, on the intracranial side of the cribriform plate (*CP*) of the ethmoid bone. The line of the transection extends from lateral (below) to medial (above). Some fascicles of olfactory axons (*A*) are spared medially. Decalcified specimen; paraffin section stained with iron-haematoxylin and van Gieson's stain. × 25.

by the cytoplasm of supporting cells, but phagocytosed inclusions were only rarely observed in the latter. Graziadei & Monti Graziadei (1979) stated that the remains of degenerated olfactory neurons were phagocytosed by macrophages within the epithelium, though their illustration (Fig. 14 of their paper) does not show a cell that resembles the macrophage of other tissues (e.g. see Carr, 1973).

The foregoing brief description of the rat's olfactory epithelium is for comparison with the epithelia of the experimental animals. A full description of the normal epithelium is given by Doucette (1981). In general, the olfactory epithelium of the rat did not differ importantly from those of other rodents as described by DeLorenzo (1957), Arstila & Wersall (1967), Frisch (1967) and Graziadei & Monti Graziadei (1979).

Transection at the cribriform plate

In the sections of decalcified specimens (Fig. 3), it was evident that the proportion of olfactory nerve fibres transected varied among animals. Sometimes almost all the olfactory nerve fascicles traversing the cribriform plate of the ethmoid had been severed, but sometimes the knife had cut only half to two thirds of them. The fibres that always escaped transection were those that entered the posteromedial aspect of the ventral surface of the olfactory bulb. Fibres entering the rostral pole of the bulb were always transected. Monti Graziadei & Graziadei (1979) have also noted that some fascicles of the olfactory nerve always escape transection at the cribriform plate.

Abnormalities were found throughout the olfactory epithelium on the operated side, from the third to the twentieth postoperative day. During the first 10 days, numerous pyknotic nuclei and empty spaces were present within the epithelium

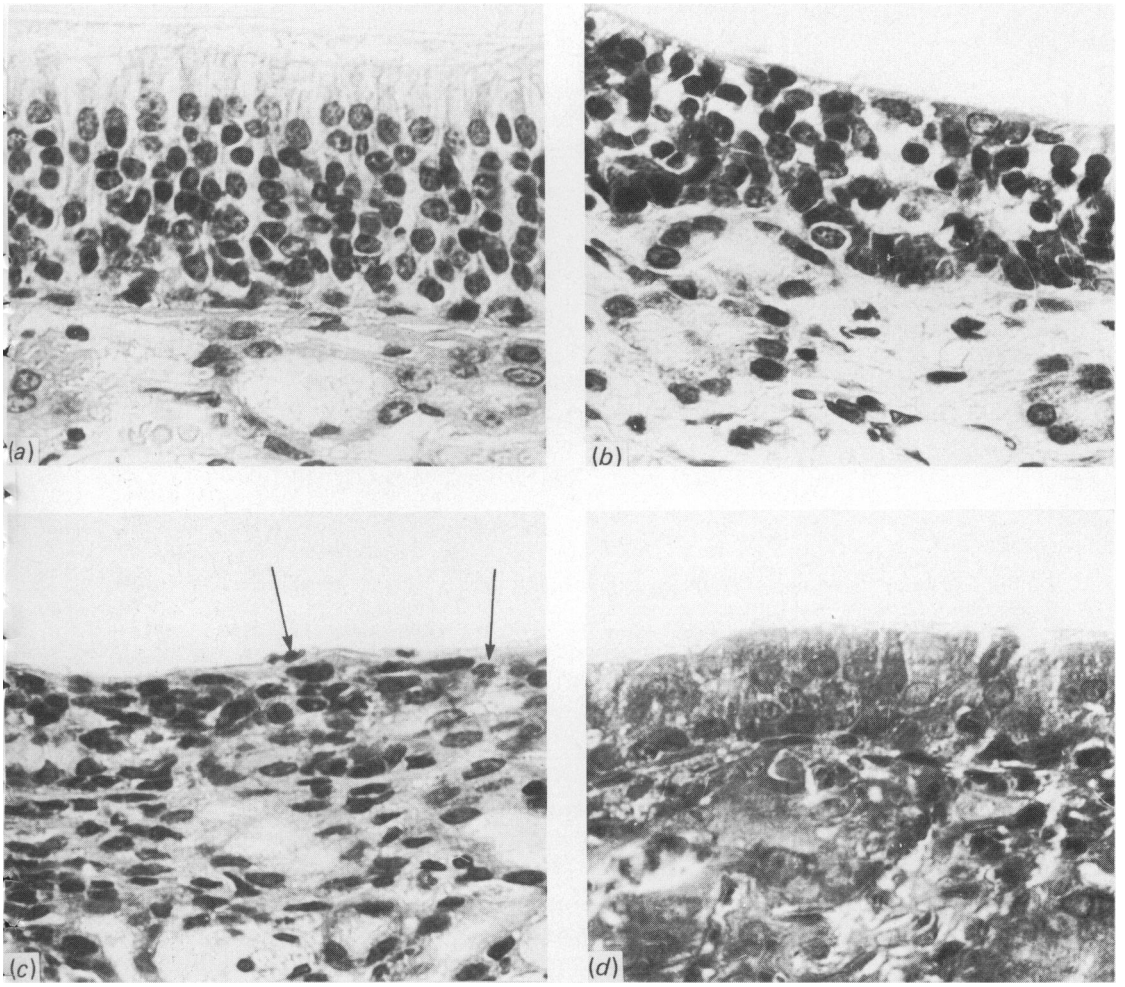


Fig. 4. Effects on the olfactory epithelium of transection of the olfactory nerves at the cribriform plate. All photomicrographs are paraffin sections stained with iron-haematoxylin and van Gieson's stain. × 650. (*a*) Olfactory epithelium of a normal rat. (*b*) 10 days after transection: the epithelium is thinner than normal and the nuclei of supporting cells are close to the surface. The epithelium also contains pyknotic nuclei and empty spaces. (*c*) Both pyknotic nuclei (arrows) and elongated nuclei with their long axes parallel to the surface are also present at 10 days. (*d*) In this animal, 5 days after transection, the epithelium is only two or three layers of cells thick, consisting of supporting and basal cells.

(Fig. 4). The nuclei of supporting cells, though not pyknotic, were abnormally close to the apical poles of the cells, and the epithelium as a whole was somewhat thinner than at symmetrical sites in the contralateral nasal cavity. The olfactory epithelium became conspicuously thinner during the second and third postoperative weeks. By the twentieth day it was usually only one or two cells thick, though still associated with conspicuous Bowman's glands in the lamina propria. Recovery then followed. The epithelium had regained its normal thickness by 31 days and remained normal at 84 days, the longest postoperative interval studied.

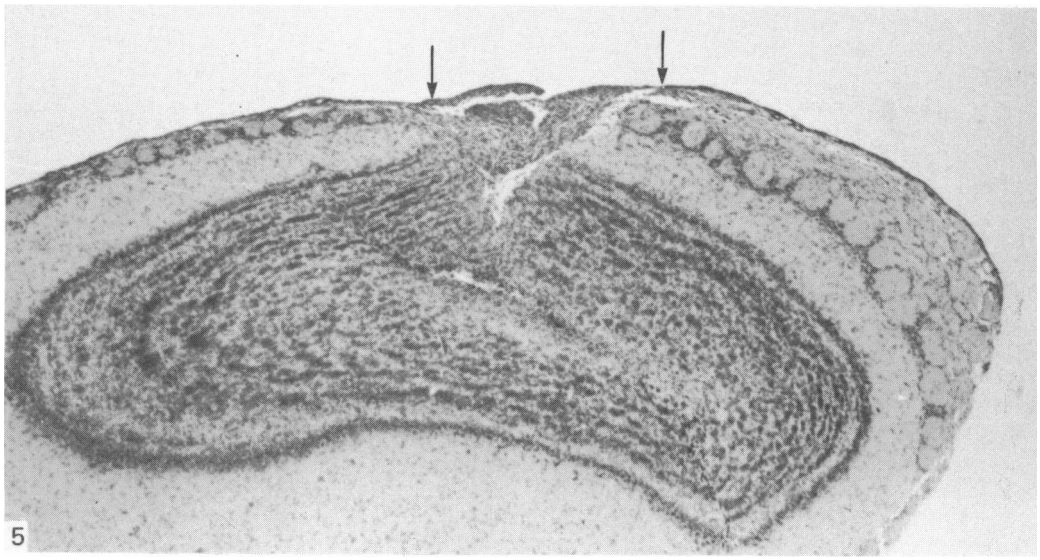


Fig. 5. Parasagittal section of the olfactory bulb of a rat in which glomeruli had been removed by suction from the dorsal surface. Arrows show the rostrocaudal extent of the lesion. It can be seen that all the glomeruli have been removed and that the lesion extends into the granule cell layer. The anterior pole of the bulb is at the right hand side of the picture. Paraffin section stained with iron-haematoxylin and van Gieson's stain. × 35.

Removal of glomeruli from olfactory bulb

Sagittal sections of the olfactory bulbs of these animals were examined to find out if the glomeruli had been successfully removed. In all cases, a lesion approximately 1·5 mm in diameter extended into either the external plexiform or the granule cell layer, thereby removing all the overlying glomeruli (Fig. 5).

Abnormalities, consisting of pyknotic nuclei and vacant spaces, were confined to the olfactory epithelium lining the roof and the dorsal part of the septum of the nasal cavity (Fig. 6). In most cases, hardly any normal receptor cells were still present in the abnormal areas.

Transverse incisions in olfactory bulb

In the sagittally sectioned, paraffin-embedded olfactory bulbs the incision invariably extended through the whole thickness of the nerve fibre and glomerular layers into the deeper parts of the external plexiform layer, and sometimes even into the granular layer. Sections from the plastic-embedded blocks of olfactory mucosa were first examined with the light microscope. Ultrathin sections for electron microscopy were cut only when abnormalities had been seen in the 0·5 μm section.

The olfactory epithelium ipsilateral to the lesion contained no abnormalities prior to the twenty-first postoperative day. At the latter time the numbers of olfactory vesicles were conspicuously reduced at the luminal surfaces of epithelium obtained from the medial part of the roof of the nasal cavity and from the adjacent most dorsal part of the septum, along the whole rostrocaudal length of the nose. The epithelium of these areas was thinner than that of the nearby lateral part of the roof, and the nuclei of the supporting cells were abnormally close to the luminal surface (Fig. 7).

Examination with the electron microscope allowed confirmation that there were

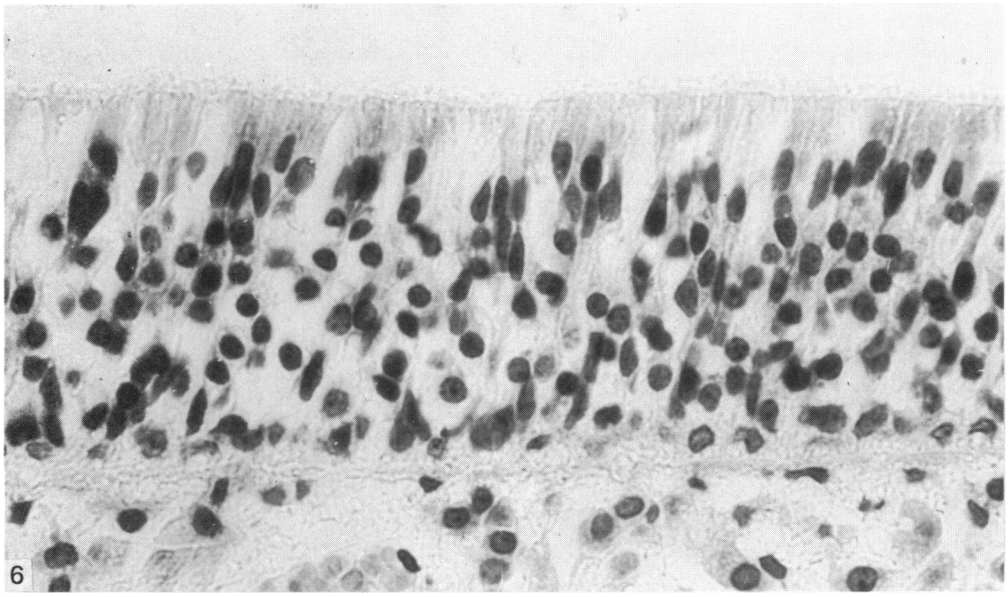


Fig. 6. Olfactory epithelium lining the roof of the nasal cavity, seven days after removal of glomeruli from the dorsal surface of the ipsilateral olfactory bulb. Pyknotic nuclei and vacant spaces are the prominent feature of this epithelium. Paraffin section stained with iron-haematoxylin and van Gieson's stain. × 700.

very few olfactory vesicles at the surfaces of the abnormal epithelia, although numerous receptor cells were still present. Evidence of degeneration (large vacuoles, multivesicular bodies and swollen Golgi complexes) was seen in some of these cells (Fig. 8). The supporting cells, recognisable by the numerous microvilli that arose from their luminal surfaces, contained hardly any cytoplasm on the apical sides of their nuclei. The nuclei of these cells were often elongated, with their long axes parallel to the surface of the epithelium. In the lamina propria beneath abnormal areas of epithelium, many bundles of olfactory axons contained large spaces, so that the remaining axons were less compactly arranged than in the normal tissue. Numerous degenerating axons were also present, and the cytoplasm of Schwann cells, which was more than normally electron-dense, contained phagocytosed debris (Fig. 9). There were some normal fasciculi, however, in the connective tissue underlying most abnormal areas of epithelium.

Abnormalities persisted in the olfactory epithelium on the operated side, even 183 days after operation. By this time, however, the epithelium was no longer abnormal along its whole rostrocaudal length. Immediately rostral to the cribriform plate the epithelium was normal, whereas further rostrally it remained abnormal along the roof of the nasal cavity and dorsal part of the septum. No olfactory vesicles were observed at the luminal surfaces of these abnormal areas even though some olfactory receptor cells were present in the epithelium.

DISCUSSION

The massive degeneration of receptor neurons, apparent as soon as three days after transection of the nerves traversing the cribrifom plate, is clearly a consequence of axonal injury. Such rapid death of neurons after axotomy is not usual in other

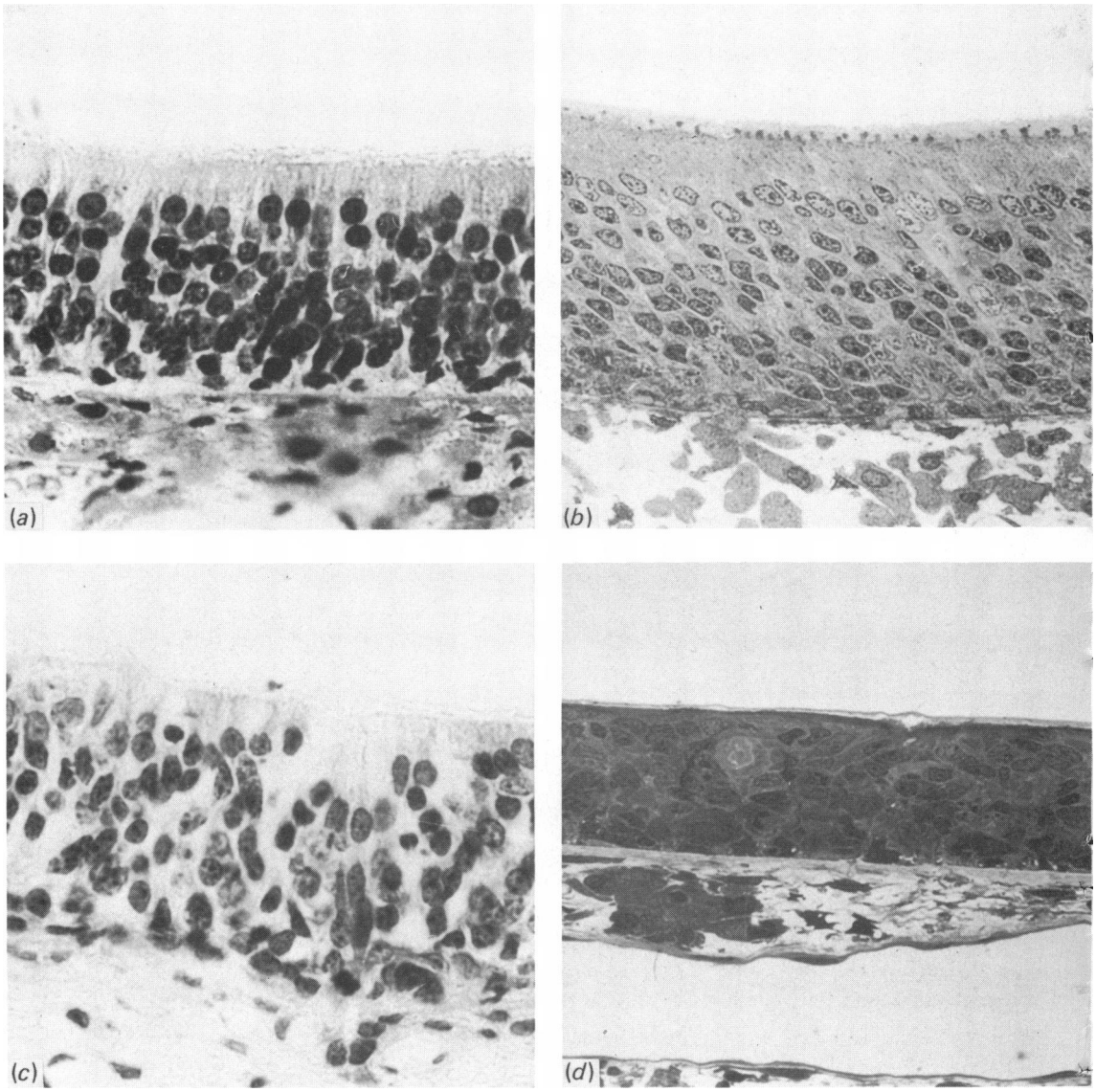


Fig. 7. The effects on the receptor cells of transecting primary olfactory axons on the dorsal surface of the olfactory bulb. (*a*) and (*c*) are paraffin sections stained with iron-haematoxylin and van Gieson's stain; (*b*) and (*d*) are semithin sections of plastic-embedded tissue, stained with toluidine blue. × 600. (Normal olfactory epithelia for comparison are shown in Figs. 1*a* and 4*a*.) (*a*) Epithelium from dorsal part of nasal septum, 14 days postoperatively, is of normal appearance. (*b*) No abnormalities are detectable on the 16th postoperative day. (*c*) The epithelium 32 days postoperatively contains pyknotic nuclei and empty spaces. (*d*) By 27 days, this epithelium is thinner than normal, there are no olfactory vesicles, and the nuclei of the supporting cells are abnormally close to the surface.

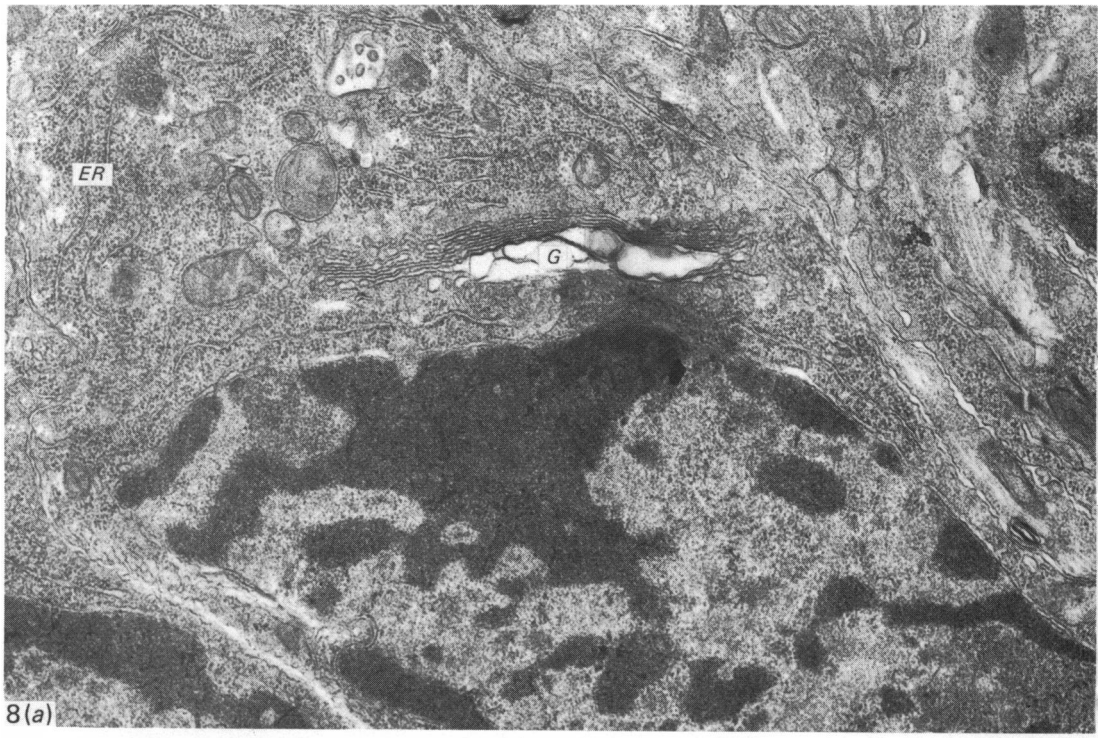
parts of the nervous system, and many types of neurons do not die even if their axons fail to regenerate (see Lieberman, 1971). The olfactory receptor neurons are exceptional, however, in that they normally have short life spans. Axonal injury evidently has metabolic effects that greatly accelerate the demise of these cells. The rat's nasal septum receives a large proportion of its blood supply from a branch of the anterior

cerebral artery that descends through the cribriform plate and might have been injured when the olfactory nerves were being cut (Hodde & Blecourt, 1979). However, closely similar changes follow extracranial transection of the olfactory nerve, which does not endanger the blood supply to the olfactory mucosa (Graziadei & Okano, 1979), in the pigeon. It is therefore most unlikely that the neuronal degeneration we observed was due to ischaemia. Similarly rapid retrograde degeneration, but in a circumscribed region of the olfactory epithelium, followed removal of the superficial layers of the olfactory bulb, thus confirming the observations of Clark & Warwick (1946) and Clark (1951). The olfactory epithelium regained its normal structure four weeks after transection of the nerves at the cribriform plate. This interval corresponds closely with the time required for re-innervation of the glomeruli of the olfactory bulb in both the rat (Monti Graziadei & Graziadei, 1979) and the mouse (Harding, Graziadei, Monti Graziadei & Margolis, 1977).

The most remarkable of our findings was that degeneration in the olfactory epithelium was delayed for three weeks following transection of primary olfactory axons on the dorsal surface of the bulb. The degenerative changes that then occurred were still present after six months, whereas the olfactory epithelium had regained its normal appearance a little over one month after cutting the nerves at the cribriform plate.

A transverse incision in the dorsum of the olfactory bulb results in complete denervation of all the glomeruli in an area up to 1·5 mm caudal to the middle third of the incision (Doucette, Flumerfelt & Kiernan, 1980). By the recognition of retrograde neuronal degeneration seen after removal of the glomeruli and the overlying layer of olfactory axons, the source of the fibres afferent to this region of the bulb was identified as the medial portion of the roof of the nasal cavity, together with the adjacent part of the septum. It was in the same part of the olfactory epithelium that delayed but prolonged changes were observed after making a transverse incision in the dorsal surface of the bulb. Re-innervation of the glomeruli caudal to the incision was first observed in the third postoperative week (Doucette *et al.* 1983), but it was not accompanied by the reappearance of normal neurons in the medial part of the roof of the nasal cavity and adjacent part of the septum. Indeed, the neurons in this area first became abnormal during the earliest stages of the re-innervation of the glomeruli. Evidently, re-innervation was by neurons in parts of the olfactory epithelium that did not formerly supply the glomeruli caudal to the transverse incision. The neurons in the epithelium that previously supplied the same glomeruli probably lived out their expected life spans. The cells of the next generation did not develop into mature receptor cells with apical vesicles. Judging by the numbers of degenerating cells in the epithelium and the paucity of olfactory axons in the lamina propria, it is probable that these abnormal neurons had shorter life spans than their normal counterparts. This supposition could be tested by using autoradiography to determine the life spans of receptor cells in different regions of the olfactory epithelium in rats injected with [^{3}H]thymidine several weeks after placement of a transverse incision in the bulb.

The delayed degenerative changes in the olfactory epithelium may have been a consequence of the failure of the axons of newly generated neurons to make appropriate synaptic contacts in the bulb. Such an occurrence is consistent with the observation (Doucette *et al.* 1983) that electron microscopy of the bulb revealed no olfactory axons passing through the site of the incision. The olfactory axons failed to grow through an incision in the nerve fibre layer of the bulb even though they



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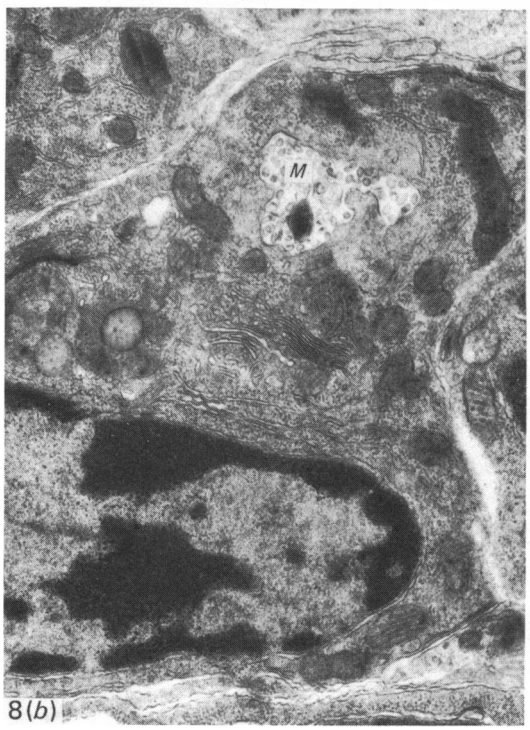

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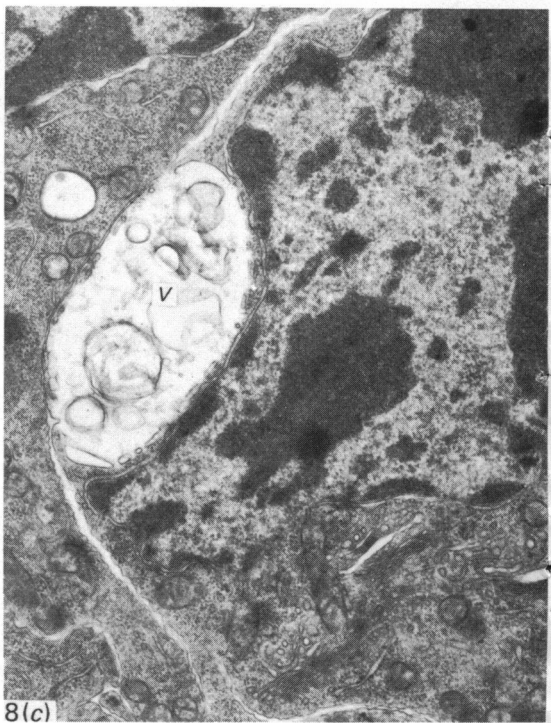

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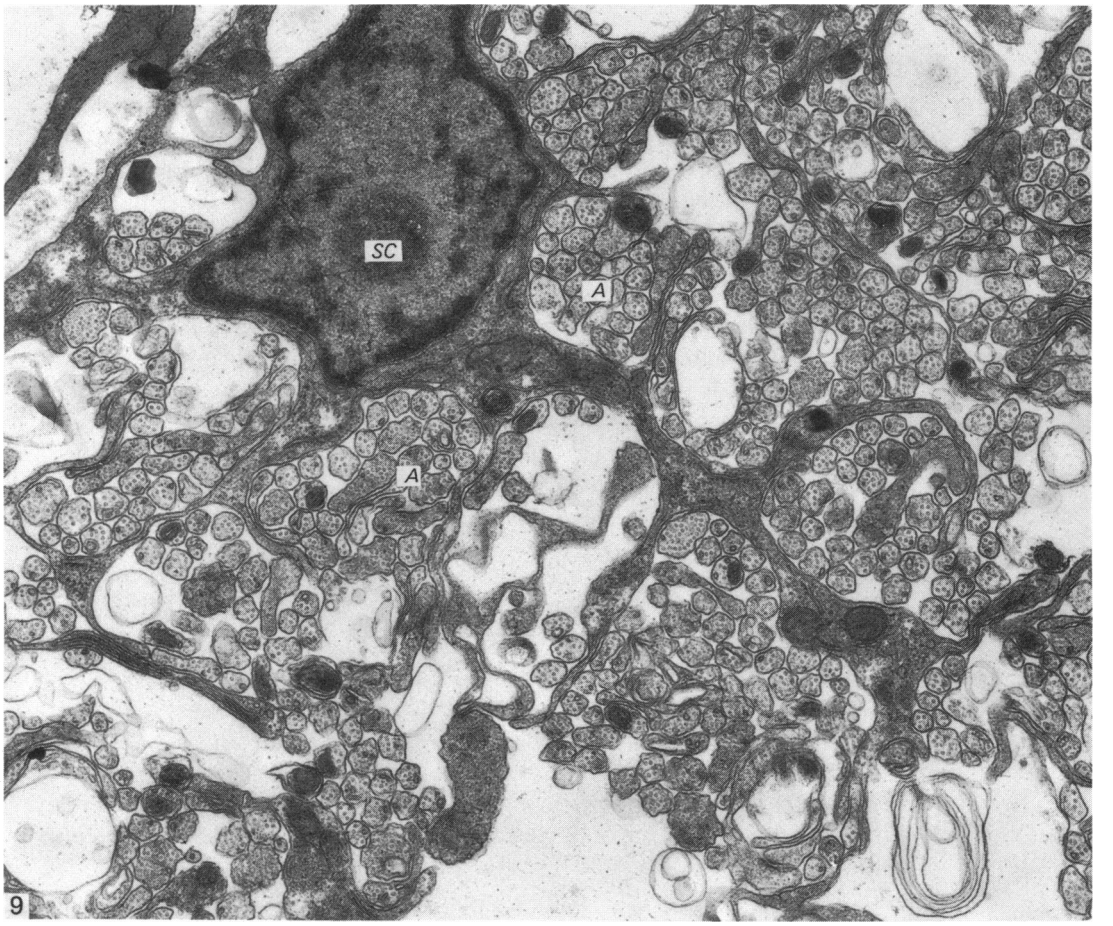

Fig. 9. Fascicles of olfactory axons (*A*) in the lamina propria underlying the olfactory epithelium, 27 days after cutting the axons on the dorsal surface of the olfactory bulb. There is much more empty extracellular space than in a normal olfactory nerve bundle. A Schwann cell (*SC*) with its basal lamina ensheaths the axons. Electron micrograph. × 8750.

could traverse a larger and apparently more damaging lesion on the intracranial side of the cribriform plate. The possible reasons for this anomaly are discussed elsewhere (Doucette *et al.* 1983). The re-innervation of the amphibian olfactory bulb has been studied physiologically by Simmons & Getchell (1981), who have observed premature death of those receptor neurons that fail to form synapses in the bulb.

Two messages are presumed to reach the olfactory epithelium after transection of

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Fig. 8. Electron micrographs showing receptor neurons in the olfactory epithelium, 21 and 183 days after transection of axons on the dorsal surface of the bulb. (*a*) This neuron, 21 days postoperatively, is recognisable by virtue of its electron-dense cytoplasmic matrix and extensive endoplasmic reticulum (*ER*). The somewhat dilated cisternae of the Golgi apparatus (*G*) and the multivesicular body in the cytoplasm at the top of the picture indicate that the cell is in an early stage of retrograde degeneration. × 23400. (*b*) This neuron also shows early degenerative change: a large multivesicular body (*M*) in the perikaryon. 183 days after operation. × 15000. (*c*) Another sign of degeneration, 21 days postoperatively, is a large vacuole (*V*) in the cytoplasm of a receptor neuron. × 16125.

axons on the surface of the olfactory bulb. The first signal must prevent the premature death of receptor neurons that would follow axotomy at the cribriform plate. It is tempting to speculate that this instruction is mediated by a substance derived from dendrites in the glomeruli near the lesion. This substance would be absorbed by the cut olfactory axons and moved by retrograde axonal transport to the somata in the nasal epithelium. Several authors have postulated that the reaction of the neuronal soma to axotomy is due to changes in retrogradely transported material (see Cragg, 1970; Kristensson & Olsson, 1974; Grafstein & McQuarrie, 1978). The postulated instructional molecules would not enter olfactory axons severed at the cribriform plate and would not be available to transected axons on the surface of the bulb after removal of a piece of tissue including the glomerular and mitral cell layers. Thus, rapid retrograde degeneration follows these two operations. Further experimentation will be necessary to obtain evidence in support of this suggestion, but it is pertinent that injured axons are known to be able to imbibe and transport macromolecules retrogradely (Kristensson & Olsson, 1974, 1976; Sparrow & Kiernan, 1979). It is also significant that in the mouse the 'olfactory marker protein', a substance found only in the cytoplasm of primary olfactory neurons, is not synthesized until these cells have established synaptic contacts (Monti Graziadei, Margolis, Harding & Graziadei, 1977; Farbman & Margolis, 1980).

When degenerative changes eventually occur in the olfactory epithelium after placement of a transverse incision in the surface of the bulb, a second instructive signal may act upon the undifferentiated basal cells to inhibit the maturation of new neurons. If, as suggested above, newly generated neurons develop abnormally and die prematurely because their axons fail to establish synaptic connections, a chemical message may be passed to the undifferentiated basal cells in their immediate vicinity. The postulated result is that the genetic expression of neuronal character by the progeny of the basal cells is suppressed, perhaps permanently, so that an epithelium devoid of mature receptor neurons is formed. Metcalf (1974) suggested that the neurons secrete a chalone which restrains the rate of mitosis of the basal cells. Death of the neurons due to transection of their axons would therefore result in an increased mitotic rate of the basal cells. Such an increase has been observed by Camara & Harding (1981) but not by Simmons & Getchell (1981). The chemical signal we propose would be concerned with the differentiation of the post-mitotic cells, irrespective of any change in their rate of production. Local effects emanating from unhealthy receptor cells may also be responsible for the abnormalities seen in the supporting cells. The loss of apical cytoplasm from these cells was conspicuous in all the olfactory epithelia that contained degenerating neurons.

Perpetuated changes in the expression of genes in populations of cells are the basis of all cellular differentiation occurring in embryonic development (see Wessells, 1977). In the olfactory system, as Graziadei & Monti Graziadei (1978*b*) have pointed out, the developmental events of neurogenesis, axonal growth and synaptogenesis occur continuously throughout adult life. It is therefore not surprising that the course of cellular differentiation in the olfactory epithelium can be changed by frustrating the normal maturation of the specialised receptor neurons.

SUMMARY

The neurons in the olfactory epithelium die after transection of the olfactory nerves intracranially, but they are soon replaced and the glomeruli of the olfactory bulb are re-innervated. It was found that, in the rat, a different sequence of events followed the transection of olfactory axons within the central nervous tissue of the olfactory bulb. The neurons in the olfactory epithelium degenerated within one week of transection of their axons at the level of the cribriform plate. The epithelium had regained its normal appearance by the fourth postoperative week. Similarly rapid degeneration, limited to a specific region of the nose, followed the removal from the dorsal surface of the bulb of a small piece of tissue consisting of afferent axons and the underlying glomeruli. We had shown previously that a transversely oriented incision passing through the nerve fibre layer immediately rostral to the same area of the bulb denervated all the glomeruli there. However, a simple transverse incision in the olfactory bulb did not produce the same retrograde changes as removal of nerve fibres and glomeruli. Retrograde degeneration following a transverse incision occurred in the expected region of the olfactory epithelium, but not until the third postoperative week. The affected area of epithelium was still abnormal after six months.

It is postulated that axotomy causes neuronal death when a trophic substance, derived from the postsynaptic components of the glomeruli, is no longer delivered by retrograde axonal transport to the perikaryon. Thus, the olfactory neuron dies promptly after axonal transection in the olfactory nerve or after removal of its terminal field of projection. When the proximal stump of its axon is near a glomerulus, after transection in the superficial layer of the bulb, the neuron still receives a trophic stimulus for the remainder of its natural life span of 30–40 days. The failure of the olfactory epithelium to become repopulated with normal neurons after axotomy within the olfactory bulb may be due to the inability of the axons of new neurons to grow across the site of the lesion. Primary olfactory neurons do not become structurally and chemically mature until they have synapsed with other neurons. The failure of the growing axons from a particular region of the epithelium to enter glomeruli may therefore result in a population of permanently immature neurons with reduced life spans.

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