ULTRASTRUCTURAL FEATURES OF TRANSNEURONAL CELL DEGENERATION IN THE OLFACTORY SYSTEM

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SUMMARY
The electron microscope has been used to study transneuronal degenerative changes in the cells of the olfactory bulb of the rabbit and rat following removal of the olfactory mucosa. The principal features of this type of change are cell shrinkage, a concentration of cytoplasmic and nuclear constituents, an alteration in the organization of nucleic acid material, and a swelling of membrane-bound systems; some elements of cellular organization appear unchanged, including synaptic specializations. The changes are described for each part of the neuron—soma, dendrite, axon and terminal—as well as the variants found in different cells. The degree of morphological alteration appears to be related to the extent of denervation for any individual cell and does not seem to be sequential. These changes are related to findings on altered impregnation properties of the same cells with the Golgi technique and are discussed in the context of the functional alteration produced by deafferentation. A new interpretation of some 'dark neurons' is also suggested on the basis of these findings.

INTRODUCTION
The studies of Wiesel & Hubel (1963a, b) on functional deafferentation have stimulated fresh interest in transneuronal degeneration by their elegant demonstration of the physiological and anatomical changes in the neurons of the lateral geniculate nucleus and visual cortex consequent on light deprivation in the kitten. There have been many light-microscopic descriptions of transneuronal degeneration using thionin-stained material (e.g. Cook, Walter & Barr, 1951; Matthews, Cowan & Powell, 1960); these have shown cytoplasmic and nuclear shrinkage and some alterations in the staining of the Nissl substance. The Golgi method has been used by several groups (Matthews & Powell, 1962; Jones & Thomas, 1962; White & Westrum, 1964; Globus & Scheibel, 1966; Valverde, 1967; Powell, 1967; Coleman & Riesen, 1968) to demonstrate alterations in the impregnation of neurons after deafferentation. There has been no complete study of transneuronal changes with the electron microscope, however, though Smith, O'Leary, Harris & Gay (1964) and Mouren-Mathieu & Colonnier (1969) have noted some features. Although the primary cause of the degenerative change following deafferentation or sensory deprivation is probably of a biophysical or biochemical nature, the latter could give rise to secondary cellular alterations, distinguishable with this technique, other than cell shrinkage.

In view of the characteristic changes in the Golgi impregnation of the constituent neurons of the olfactory system after olfactory nerve section, this site is very suitable...
A. J. Pinching and T. P. S. Powell

for studying the ultrastructural correlates of these altered impregnation properties; the findings with the 2 techniques are complementary and their correlation may throw some light on the possible mechanisms of Golgi impregnation. The electron-microscopic changes that were found seemed to correspond to some previous descriptions of 'dark neurons' in the central nervous system (Cammermeyer, 1962; Cohen & Pappas, 1969), so that this study has taken on a further significance in the elucidation of some of the different types of 'dark neuron' and their origin. This work has been done in the course of an analysis of the structure of the olfactory bulb in this laboratory (Price & Powell, 1970-1972; Pinching, 1970).

MATERIAL AND METHODS

Electron microscopy

The olfactory mucosa was destroyed unilaterally in 14 rabbits and 18 rats, aged from 5 to 7 weeks, under Nembutal (rabbits) and Avertin (rats) anaesthesia. They were allowed to survive for periods of 10–90 days (rabbits) and 10–250 days (rats), a pair at each survival time. Perfusion was carried out under anaesthesia and hypothermia (20–25 °C) with a mixture of glutaraldehyde (1 %) and formaldehyde (4 %) in phosphate buffer (0·1 M) at pH 7·3, following a brief wash-out with buffered salt solution. After a few hours the brain was removed from the cranial cavity and left in fixative for a minimum of 3 h; blocks of olfactory bulb and pyriform cortex were taken from both sides and washed in phosphate buffer with 10 % sucrose overnight. These blocks were post-fixed in osmium tetroxide (2 %), dehydrated in alcohol and embedded in Araldite. Thick sections (2 μm) were cut and stained with a mixture of methylene blue and Azure II (Richardson, Jarett & Finke, 1960) to show the layers of the bulb; ultrathin sections were taken from the blocks and stained with uranyl acetate and lead citrate. Most of the material was examined in serial sections, which were mounted on formvar-coated slit grids.

Light microscopy

The same operation was carried out on 12 rabbits and 7 rats and these were allowed to survive for the same periods as for electron microscopy in both species. The brain was then exposed under anaesthesia, the animal decapitated and its skull placed in Golgi-Cox fixative; after 1 h the brain was removed from the skull, cut into blocks and placed in fresh Golgi-Cox fixative. These were left for 8 weeks, then embedded in L.V.N. and cut at 150 μm; these sections were processed according to the method given by Sholl (1953).

RESULTS

Rabbit

Light-microscopic studies on transneuronal degeneration have concentrated largely on size changes of cells, their nuclei and processes; the electron microscope, while in general unsuited to this kind of study on account of the thinness of the sections used and the limited areas studied, can provide more detailed qualitative data on the cytological changes involved. It is possible that the changes to be described here represent only one feature of the transneuronal degenerative process, being at this level the most distinct morphologically; however, the regularity of this characteristic degenerative change and the gradation in its severity found in different cells, as well as the close correspondence between the proportion of affected cells and the extent of denervation within each brain, indicate that it is directly consequent upon the section of the
Transneuronal cell degeneration in the olfactory system

Olfactory nerves. Subtler alterations may occur in cells which appear normal in this kind of examination. Thus, although the published quantitative results of Matthews & Powell (1962) on mean cell areas would indicate that all mitral and tufted cells shrink, the areas of some individual cells remain within the normal range after degeneration. The electron-microscopic findings show that roughly two-thirds of the mitral and tufted cells show the qualitative changes described below, while the rest appear normal, although they may be slightly shrunken.

The cellular changes described here have been seen at all the survival times studied and, apart from slight variations due to differences in the extent of denervation, there appears to be no significant alteration in the proportion of cells affected after 24 days. In all the material used, both from the normal and the operated sides, the material was regularly well fixed and the cells that remained normal were well preserved. However, because the onset of transneuronal degeneration is accompanied by a marked increase in the extracellular and glial volume in the glomeruli (Figs. 17, 18, 22), although the processes that remain seem adequately preserved, most material was taken from the deeper layers. In animals of 10-49 days' survival (aged 60-95 days), few or no cells on the normal side showed any cytological change similar to that on the operated side, and those that did could be related to some olfactory nerve degeneration at glomerular level, either spontaneous or possibly due to slight damage of the nasal septum at operation. In the older animals of 70 and 90 days' survival (aged 120-135 days), more changed cells were seen on the normal side, although they were still considerably rarer than on the operated side; this would correspond with the observation that atrophic rhinitis occurs in animals of this age (Matthews & Powell, 1962).

While all the features described have been found in mitral, tufted, periglomerular, granule, and occasionally, short-axon cells, and show no variation according to type, for the reasons stated above, the mitral and granule cells will be used principally as examples; the mitral cells have the added advantage that they may be readily distinguished from glial or other cells by virtue of the reciprocal synapses on their cell body and dendrites (Andres, 1965) (Figs. 12, 15). It should be noted that sections from the normal and operated sides were carefully examined in each animal and were directly compared in the initial analysis. The typical features of the degenerative changes will be described for each part of the neuron, after which the variations found in more or less severely affected cells and the time of onset of each change will be indicated.

**Soma.** The most striking features shown by transneuronally degenerating neurons are an increased electron density, apparently due to a concentration of the cytoplasmic and nuclear contents, and the loss of distinction between different parts of nucleus and cytoplasm (Figs. 1, 3-7). The perikaryon regularly appears very shrunken in comparison with neighbouring, apparently normal cells, as well as with those of the normal side. Unlike the smooth outline of normal cells (Fig. 1), the boundaries of these cells are irregular, as if they had been scalloped by adjacent processes (Figs. 3-6). The nucleus also appears somewhat shrunken, the nuclear membrane often being detached and vacuolated in between the pores which usually remain intact, thus giving rise to a beaded appearance (Figs. 3, 7). It should be noted that the normal
mitral cells of the rabbit, unlike those of the rat, have marked nuclear indentations, and these remain despite the transneuronal change. The nuclear contents, which are normally clumped into islets of granular material, become densely packed and tend to lose all such local morphological differentiation (Figs. 6, 7). The nucleolus however, as far as we are able to assess, seems unchanged in all but the most extreme cases; in a few very dense cells, the nucleolus does show a segregation of its granular and fibrillar components (Fig. 9) similar to that seen with actinomycin D treatment of kidney and liver cells (Bernhard & Granboulan, 1968). Intranuclear fibrillar rodlets (Masurovsky, Benitez, Kim & Murray, 1970) have been found in tufted and periglomerular cells more frequently than in normal material (Fig. 8).

Cytoplasmic contents are tightly packed within a grey granular background, which seems to consist of a highly concentrated form of the fine dense material that is normally present in the cell (Figs. 3–7). Free ribosomes, lending a coarse grain to the cytoplasm, are present in greater proportion, while rosettes are rarely encountered; the granular endoplasmic reticulum seems to retain its attached ribosomes. All membrane-bound systems except lysosomes—that is, mitochondria, endoplasmic reticulum, Golgi apparatus and multivesicular bodies—have a tendency to be swollen or vacuolated, the mitochondria showing a characteristic ‘blown-up’ appearance (Figs. 3, 4, 6, 7), which is most marked in the large mitral cells; normal mitral cells tend to show some blown mitochondria, but the disruption is more regular and more severe in transneuronally degenerating cells. Golgi apparatus and endoplasmic reticulum (Figs. 3, 6, 7) are found widely dilated in a manner never seen in normal cells. Fine granular lysosomes (Fig. 7), though present in normal neurons, are seen more frequently in degenerating cells, according to the severity of the change, but are never strikingly in evidence; dense lysosomes do not show any increase or morphological change. Large empty vacuoles often appear in the cytoplasm, particularly adjacent to the plasma membrane (Figs. 3–5). Synaptic structures will be dealt with in the following section.

Dendrites and appendages. Essentially the same kind of degenerative change has been found in the dendrites of the affected cells, including the peripheral processes of the granule cells, and if the cell body is affected then its processes are altered with a corresponding severity (see Figs. 2, 3). In the main dendritic shafts, which generally appear shrunken, there is an increase in the fine granular background of the cytoplasm as well as in the proportion of free ribosomes, while rosettes are rare (Figs. 2, 3, 5, 12–15, 19, 20); mitochondria and endoplasmic reticulum are swollen or dilated, as is any Golgi apparatus that may be present in the initial parts of dendrites (Figs. 2, 3). But particularly characteristic of the dendritic change is the dense packing and altered appearance of the neurotubules (Figs. 2, 12, 19, 20); in severely affected cells they may be so densely packed that the intertubular spaces are no wider than the diameter of the tubules. The tubular outline, meanwhile, becomes more clearly defined and loses the web-like fringe that it normally exhibits; it may be that this fringe constitutes part of the fine granular background in dendrites. The clearer outline may be due to some swelling of the tubules in degeneration; however, as shown in Figs. 19 and 20, it is caused primarily by the fact that while the external tubular diameter
remains approximately constant, the internal diameter increases. Fine granular lysosomes are a little more common than usual. Varicose dendrites or varicose portions of dendrites shrink in such a way as to retain their varicose character, the connecting portions becoming extremely attenuated and containing little more than tubules, and the varicosities remain comparatively paler, though revealing a typical cytoplasmic concentration (Fig. 18).

In the glomeruli, the olfactory terminals that have degenerated in the first 10–18 days after mucosa removal (Fig. 13) leave the post-synaptic thickening and extracellular material exposed, as described in the rat (Pinching, 1969); degenerating dendrites with post-synaptic specializations persisting have been found in the glomeruli of the rabbit (Fig. 14), but these are relatively rare. This paucity of membrane thickenings seems to be a reflection of the atrophy of the distal portions of the glomerular dendrites, which are only rarely encountered in material of more than 18 days' survival, being replaced by fine dark threads of cellular material (Figs. 17, 18); these occasionally show signs of internal structure such as mitochondria or tubules but do not reveal any distinguishable post-synaptic structures.

In both somata and dendrites of all the transneuronally degenerating cells we have observed, regardless of the severity of the change, synaptic specializations in relation to normal terminals or gemmules remain intact and unaltered. The reciprocal synapses of the mitral and tufted cells show exactly the same structure and arrangement as they do in normal material, including the presynaptic aggregation of spherical vesicles on the mitral/tufted cell side, vesicles that are in all respects normal, without any tendency to swell or to disperse into the surrounding cytoplasm (Figs. 12, 15); the post-synaptic thickening of the gemmule to soma/dendrite synapse is unchanged, while the extracellular material and the cleft width of both synapses are the same as in normal material. Similarly all normal axon terminal synapses on to the dendrites and somata of any type of transneuronally degenerating cell are structurally unaltered. There is no evidence in our material of any change in the structure or staining properties of the unit membrane of degenerating neurons (compare Kruger & Hamori, 1970).

Spines and gemmules show the same gradations of change as the processes from which they arise (Figs. 5, 6, 10, 21, 25), and their pedicles shrink down to fine threads of electron-dense material (Figs. 6, 10, 22). Ribosomes and vesicles (where present) become concentrated and the granular background becomes denser and less sparsely arranged; mitochondria, which are often found in these appendages, are sometimes distorted (Fig. 10), though spine apparatus seems relatively resistant to dilatation (Fig. 25). Synaptic specializations are unchanged both in reciprocal synapses (gemmules) (Fig. 21) and in spines post-synaptic to normal axon terminals (Fig. 10). In a few cases, the gemmules swell and take on a watery appearance (Fig. 22), and in these many of the vesicles also swell; this has generally been found in conjunction with an extremely attenuated pedicle and may represent the degenerative isolation of the appendage from the parent dendritic shaft.

Similar changes have been found in somata, dendrites (Fig. 23) and spines (Fig. 27) of cells of the ipsilateral pyriform cortex in material of 49 days' survival and over. Dendrites and spines of the cortical neurons appear to degenerate without these...
changes necessarily affecting their parent stem dendrites or cell somata. The changes
are sparse in comparison with those in the bulb but represent a transneuronal de-
geneneration occurring across 2 synapses, as is presumably the case with the granule
and short-axon cells, neither having primary contact with the incoming olfactory
axons. Degenerating spines and dendrites are sometimes found receiving synapses
from apparently normal terminals (Fig. 23), indicating that the degenerative change
in this region spreads along affected dendrites.

Axon hillock and initial segment. The axon hillock and initial segments of the axons
of normal mitral, tufted and short-axon cells are very similar to those described by
Palay, Sotelo, Peters & Orkand (1968) and Peters, Proskauer & Kaiserman-Abramof
(1968), while those of the small periglomerular cells show less clear differentiation of
these regions. The axons of transneuronally degenerating mitral and tufted cells
(Fig. 11) show a concentration of cytoplasmic constituents, similar to that described
for the cell somata and dendrites, with the result that it is difficult to be sure of any
dense plasma membrane undercoating or tubular aggregation because of the in-
creased cytoplasmic granularity and the dense packing of the neurotubules. How-
ever, neither of these features is as marked as in normal cells and may even be absent,
as is the extracellular granularity described by Peters et al. (1968). The identification
of the axon hillock and initial segment was therefore only certain in cases where the
direction of the process was unequivocally that of an axon of these cells, and where
serial sections revealed no reciprocal synapses on to the supposed initial segment. It
has not been possible to trace one of these axons as far as the beginning of the myelin
sheath. Free ribosomes, dense-cored vesicles and clear vesicles are common, as in
normal initial segments, though the former are rarely clustered in transneuronal de-
geneneration; mitochondria and other membrane-bound organelles are swollen and
disrupted. The neurotubules appear slightly swollen and stand out in a characteristic
manner; synaptic structures, where these occur, are unaltered. It has not been possible
to make unequivocal identifications of the axon hillocks or initial segments of tran-
sneuronally degenerating periglomerular or short-axon cells; granule cell peripheral
processes may be considered as dendrites for the purposes of this study.

Axon and terminal. The axons of mitral and tufted cells, after running in the lateral
olfactory tract, terminate in the pyriform cortex (Cajal, 1955; Westrum, 1966), but
before leaving the bulb they give off collaterals which terminate in the granule cell
and external plexiform layers (mitral and tufted cell collaterals) and in the peri-
glomerular zone of the glomerular layer (external tufted cells) (Cajal, 1955). A pro-
portion of the myelinated axons of these cells in the olfactory bulb, lateral olfactory
tract and in the pyriform cortex are altered at survival periods of 33 days and over
(Fig. 24). They present a shrunken appearance and the cytoplasmic density increases,
and tubules and filaments become more concentrated, lending a characteristic grain
to these processes, similar to that described for early orthograde degeneration; mito-
chondria and multivesicular bodies swell and become disrupted. Occasionally these
axons contain large vacuoles and dense lysosomes; free ribosomes are common,
though they are rare in normal myelinated axons. In severely affected axons the
cytoplasm consists entirely of a granular osmiophilic matrix with glycogen-like
Transneuronal cell degeneration in the olfactory system

granules, and the whole axon may be enclosed by a glial process showing reactive changes.

The processes that had been tentatively identified as axon collateral terminals from the examination of normal rabbit and rat material, as well as in relation to experimental studies in the rat (Price & Powell, 1970c; A. J. Pinching, unpublished observations), were observed to alter in appearance in a similar manner to that shown by their supposed parent cells in undergoing transneuronal degeneration (Figs. 25, 26). These morphological changes are quite distinct from those occurring in terminals after orthograde degeneration but, being subtler in nature, might have been considered artifactual or within an acceptable normal range. A double-blind trial was therefore carried out on the plexiform layer of the pyriform cortex: unlabelled sections from several blocks of ipsilateral and contralateral pyriform cortex from each brain were examined in random order and identified on the basis of axon terminal changes in this layer. The trial showed that the cortex from each side could be distinguished on this basis and that the axon terminals in the pyriform cortex do undergo characteristic changes, identifiable from 33 days after olfactory nerve section (Fig. 28). It is worth noting that the only block about which identification was uncertain was from a 70-day animal in which the spontaneous degeneration in the bulb of the normal side was quite marked.

The transneuronally degenerating terminals show a variable degree of shrinkage and often take on an irregular outline; the vesicles in these terminals become crowded in the fine granular cytoplasm and lose their clearly defined boundaries, seeming on occasion to merge with the increased background granularity (Figs. 25, 26, 28). This change in texture is the most evident and the most constant change in the axon terminals of these cells, but where mitochondria are present they often swell (Fig. 25); some vesicles and cisternae also swell and become vacuolated (Fig. 28). In extreme cases the background becomes entirely opaque and glial processes may invaginate the terminal; the terminal and its vesicles then take on very distorted shapes. In other processes, dense granules are seen which may correspond with the glycogen granules seen in glia; free ribosomes are also occasionally found (Fig. 25). Synaptic thickenings remain unchanged in both pre- and post-synaptic processes at the survival periods studied, and there is no evidence of the complete removal of these terminals by the presence of bare post-synaptic thickenings. Where the preterminal axon is found in continuity with the terminal, it is very attenuated, containing little else but tubules, and has an irregular, wavy appearance.

These features of transneuronal degeneration of axons are the same in all parts of the olfactory bulb where collaterals terminate, as well as in the superficial half of the plexiform layer of the pyriform cortex, though the terminals in the latter show rather more mitochondrial and vesicular swelling. The axon terminals of periglomerular cells undergo the same type of change (Fig. 11), while the granule cells have no typical axon (Cajal, 1955; Price & Powell, 1970a); so few degenerating short-axon cells have been found that it has not been possible to identify axon terminal changes for these cells, although it may reasonably be presumed that they are similar to those described above.
Mild and severe forms of degeneration. To avoid the possibility of comparing different portions of cells in different planes of section, the variation in degeneration will be described in cell somata, although their dendrites and axons show similar variations. All survival times present a range of transneuronally degenerating cells, some of which show only slight differences from normal cells, others being severely altered; the majority, however, correspond with the above description of moderate degeneration, having only slight variations in density. This gradation of change may either represent an ongoing alteration over the survival periods studied, some cells changing at a greater rate than others, so that all types are seen at any time, or alternatively the degree of change in a given cell is a reflection of the extent of its deafferentation, so that partially deafferented cells show less severe changes than those that are totally deafferented. The evidence gained from the survival times covered in this study would tend to support the latter explanation. The degenerative change does not appear to spread from a particular part of the soma or dendritic tree, but all parts of a given neuron are affected with a corresponding severity, though the axon terminal changes may be delayed (see below).

The characteristics of mild degeneration (Figs. 4, 5) are a slight shrinkage of the perikaryon and a slight increase in the background granularity, as well as mitochondrial swelling and a tendency for pale vacuoles to appear near the plasma membrane; an increased proportion of free ribosomes is also common. Endoplasmic reticulum and Golgi apparatus may show no change and, though there is some loss of differentiation within the nuclear material, the nuclear membrane remains attached and the nucleolus appears normal. As the density of the granular background increases, so the cytoplasmic and nuclear alterations appear or become more marked until the typical appearance is achieved (Fig. 3).

The most severely affected neurons are extremely electron-opaque in both nucleus and cytoplasm, the latter to such an extent that at low magnification it is sometimes difficult to distinguish the boundary between it and the nucleus. These cells may take on a stellate appearance (Fig. 6) by virtue of a marked indentation by the adjoining profiles and the presence of widely dilated vacuoles next to the plasma membrane. The swelling of membrane-bound systems is often absent, but instead they take on distorted shapes, though mitochondria retain their blown-up appearance. It is only in such severe cases of transneuronal degeneration that any gross segregation of nucleolar components into granular and fibrillar portions occurs (Fig. 9), indicative of a degenerative change in this organelle (Bernhard & Granboulan, 1968).

At most survival times, a small number of cells are found to have the characteristic changes of membrane-bound systems, but show no sign of granularity or concentration in their cytoplasm; sometimes the cytoplasm, particularly of the larger dendrites, appears diluted, dendritic tubules becoming considerably more widely spaced than usual. The ribosomal rosettes of these cells are generally intact. Their nuclei often appear shrunken and the nuclear membrane has a marked tendency to vacuolate. These cells, which are not found on the normal side except occasionally in older animals, may represent a different type or stage of transneuronal degeneration; they
Transneuronal cell degeneration in the olfactory system
could arise, however, as a consequence of an altered composition of the extracellular
fluid.

Time course of the cellular changes. Up to 10 days after olfactory nerve section, the
terminals of these axons degenerate; at 18 and 24 days the olfactory nerve terminals
have disappeared and the glomerular arborizations of the dendrites of mitral, tufted,
and periglomerular cells are shrunken and atrophied, and their parent cells show the
characteristic degenerative changes in their main dendritic shafts, somata and axon
initial segments. By 33 days the axons of these cells show clear changes in the olfactory
bulb and pyriform cortex, although some collaterals in the former are seen to de-
generate earlier, perhaps representing those with shorter axons (Fig. 25). Granule
cells are seen to degenerate at the same time as the other bulbar cells but dendritic
changes are not seen in the pyriform cortex until 49 days after mucosa removal. This
difference may reflect the additional connexion between mitral/tufted cell dendrites
and the granule cell peripheral process, while the pyriform cortical neurons have only
an axonal input from these relay cells; it indicates that the cortical cells are changed
only after the appearance of morphological changes in the terminals of their afferent
input and could result from these.

Glia and extracellular space. The glia of the deep layers of the olfactory bulb show
little sign of active involvement in transneuronal changes, except in cases of severe
degeneration of terminal processes, while those of the glomeruli, in addition to this,
are seen to participate only in relation to the removal of olfactory nerve terminals
(Fig. 13). However, the volume of the glial cytoplasm of the glomerular layer, and to
a much lesser extent of the deeper layers, increases; this consists mainly of pale
watery cytoplasm, often similar to extracellular space but for the sparse reticular
background, the occasional mitochondrion or other inclusion, and the surrounding
plasma membrane (Figs. 17, 18, 22). These glial processes may be acting to ‘fill in’
the spaces created by the loss or atrophy of the olfactory nerves and dendritic terminals
of the cells with glomerular arborizations, possibly having taken up the fluid lost from
these degenerating neurons. The less marked glial changes of deeper layers may
reflect the qualitatively and quantitatively less severe degenerative changes in these
layers. Similarly, there is an increase in the extracellular space in the glomerular layer
and the superficial part of the external plexiform layer, particularly around the fine
threads that are the remnants of the dendritic arborizations (Figs. 17, 18). The changes
in these elements of the neuropil may either represent the situation in the living
animal or else a change in their composition and in the fluid balance between them
and the neuronal processes, as revealed by their altered properties in the presence of
fixative. This paradoxical finding of increased glial and extracellular volume in the
shrunken olfactory bulbs seems to reflect the discrepancy between the neuronal
shrinkage and the shrinkage of the bulb as a whole (cf. Matthews & Powell, 1962).

Rat

In rat material of 10–150 days’ survival, apart from very occasional individual
processes, no transneuronal change is seen; the post-synaptic processes retain their
morphological integrity and are packed in the glomeruli without abnormal extracellular
Many post-synaptic thickenings without pre-synaptic specializations are found on dendrites and are apposed to other dendrites as described previously (Pinching, 1969). At 200 days a few glomeruli show transneuronal changes, so that adjoining glomeruli are found with the typical appearance found in the rabbit glomeruli under these conditions on the one hand, and the typical appearance of earlier long-term rat glomeruli on the other. The alterations in the dendrites and somata of all cell types are exactly comparable to those described above for the rabbit (Fig. 16), showing similar gradations in severity and with the same glomerular appearance. The post-synaptic thickenings in the glomeruli decrease in number and this may again be related to the degeneration of the fine terminal portions of the glomerular dendrites. No sign of transneuronal change has been found in the terminals of the axon collaterals of the mitral and tufted cells in this species, but this is probably due to either the low sampling of a small degenerating population or the fact that the cellular change has not yet reached the axon terminals.

Qualitative light-microscopic changes

In studying 2-μm sections, stained with methylene blue and Azure II (Richardson et al. 1960), for the orientation of blocks of this material for ultrathin sectioning, it was noticed that a variable number of the mitral, tufted, granule and periglomerular cells on the ipsilateral side were stained deep blue throughout their nuclei and cytoplasm, and seemed shrunk in relation to neighbouring cells. These were most evident in those parts of the bulb where denervation had been most complete (anterior and medial). These are the same cells that appear electron-dense, and show the same gradation of change, as determined by the study of sections with both methods, taken within 1 μm of each other (cf. Cammermeyer, 1962; Cohen & Pappas, 1969).

Golgi–Cox results

The same span of survival times have been studied in the rabbit as were used for electron microscopy. As described previously (Matthews & Powell, 1962; Powell, 1967), the cells of the olfactory bulb undergoing transneuronal degeneration are resistant to impregnation after the removal of the olfactory mucosa. At 10 days there are fewer cells impregnated than on the normal side, but there is a reasonable proportion of all types. At 17 days the appearance of the 2 sides is strikingly different, with very few typical mitral, tufted or periglomerular cells stained and the granule cell population somewhat reduced; this situation persists unchanged up to 90 days. These authors also noted that several unusual cells appear in the mitral cell layer at the longer survival times; similar ones have also been found in the external plexiform layer. These have a rather stellate, but often indeterminate, form and seem at low magnification to have fine strands running from them in 2 primarily radial bundles, of deep and peripheral orientation. A careful examination of these bundles at higher magnification shows that many of the finer processes are spiny, while others are varicose. Some cells are found with cell bodies very similar to normal mitral cells, if a little shrunken, and very occasionally these show several typical secondary dendrites in addition to the plethora of fine processes that surround them. After examination of
Transneuronal cell degeneration in the olfactory system

electron-microscopic material from rabbits of the same survival times, the altered cells have been identified as degenerating mitral and internal tufted cells with their shrunken axons and dendrites, regularly surrounded by the peripheral processes of a group of granule cells lying deep to them, the cell somata of which may often be seen in continuity with the strands deep to the mitral cells. No glial cells additional to those normally present in the mitral cell layer have been found with the electron microscope that could correspond to these Golgi-stained cells, while the indented or stellate appearance of the degenerating mitral cells is strongly suggestive that these are the impregnated cells.

As noted by Powell (1967), the neurons of the pyriform cortex at the longer survival times (49 and 90 days) showed a distinct thinning of their dendritic branching, particularly in the superficial part of the plexiform layer, and a slight reduction in the number of cells stained. The former effect corresponds with that described by Jones & Thomas (1962) after removal of the olfactory bulb. There was no clear evidence of a reduction in the number of spines on the remaining impregnated dendrites on the operated side (cf. White & Westrum, 1964), but the material was not adequate for a detailed study of the spines in this region.

All the differences between the operated side and the normal side of Golgi-stained material are distinct and occur regularly in all the brains studied; they cannot be discounted as reflecting the variability of the technique in view of their entire correspondence with electron-microscopic observations, as well as the sensitive way in which they reflect variations in the extent of denervation.

Studies on the rat with the Golgi-Cox method at short survival times after mucosal removal (10–100 days) show none of the qualitative changes seen in rabbits of the same survival periods. However, changes similar in all respects are seen in material of 250 days' survival, thus confirming the electron-microscopic observations of the onset of transneuronal degeneration in the rat at 200 days' survival.

DISCUSSION

The material

The olfactory bulb of the rabbit has proved to be a suitable site for the study of transneuronal cell changes at the electron-microscopic level: the single high-density afferent pathway which may be interrupted without opening the cranial cavity, together with the well-defined laminar structure of the bulb, whose cell types may be readily identified, make this an excellent preparation. Although the use of young animals has largely avoided the complication of atrophic rhinitis and subsequent transneuronal changes on the control side, the limited appearance of such changes in the control side of older animals only, tends to confirm rather than confound the suggestion that the type of change described does represent true transneuronal degeneration.

The term 'dark cell' has been avoided in this paper since its wide use has led to some confusion in the literature between quite different dark cell types in the CNS, both normal and pathological (see Cammermeyer, 1962; Cohen & Pappas, 1969);
since the electron microscope can provide information only on electron density variations, to consider all cases of increased density or darkness as a fixation artifact, or as representing a non-specific pathological change, precludes the proper analysis of the detailed characteristics of different dark cell types. Though poor fixation may give rise to a certain type of dark cell or dendrite, their regular presence cannot be so discounted in material that is on all other criteria well fixed. In the brains used for this study, fixation was of a good standard and the 'dark cells' interpreted as transneuronally degenerating neurons were distributed in a manner closely related to the extent of the denervation in any part of the bulb. Although the dilatation and disruption of membrane-bound systems within the affected cells is likely to have occurred during fixation, it is specific to the degenerating cells and this suggests that it is a reflection of an increased fragility or susceptibility of these systems to the fixative, perhaps as a result of the altered osmotic properties of their cytoplasmic environment. In the rat material of 200 days' survival only a few glomeruli show changes; thus glomeruli adjacent to those undergoing transneuronal degeneration and subjected to exactly the same experimental treatment, show no evidence either of poor fixation or transneuronal change, but only the characteristic appearance of rat material of shorter survival periods.

The glial involvement in the degeneration of the terminal dendritic and axonal processes of some cells is strong evidence that the changes described are not simply produced at perfusion, as is the cell specificity of the change and its occurrence throughout the processes of any involved cell in the olfactory bulb. Cell death may be ruled out in the vast majority of cases, on account of the retention of certain features of cytological organization, such as the granular endoplasmic reticulum and the vesicle clustering adjacent to the presynaptic part of dendro-dendritic synapses (Rall, Shepherd, Reese & Brightman, 1966). Apart from one clear example in a brain rejected from this study, there was no evidence of direct injury to the olfactory bulbs, either through the cribiform plate at operation or at any other stage. Since the blood supply of the olfactory bulbs is separate from that of the olfactory mucosa, changes due to a vascular lesion may be discounted, as may any question of infection; furthermore, the appearance of the bulbs is quite different from that found in ischaemia or after local bulbar lesions. We feel justified therefore in the conclusion that the changes described here represent true transneuronal degeneration, if only certain features of it. Mouren-Mathieu & Colonnier (1969) have identified similar changes in the Purkinje cell dendrites of the cerebellum after interruption of parallel fibres as transneuronal degeneration; swelling of membrane-bound organelles in the lateral geniculate nucleus was described by Smith et al. (1964) from 7-100 days after eye removal.

Possible origins of the morphological changes

Most of the observed cytological alterations are explicable in terms of a concentration of the cellular constituents, consequent on or giving rise to cell shrinkage. The changes in the distribution and arrangement of the nucleic acid components of both nucleus and cytoplasm are the only clear evidence of a modification of cellular organization and activity (see Brattgård, 1952). It could be considered that the increase in free
Transneuronal cell degeneration in the olfactory system

Ribosomes is a reactive change (Kruger & Hamori, 1970) or else an indication of a reduction of cellular activity to a basal level. The apparent increase in neurotubules in dendrites and axons could similarly be considered as a reactive generation of these structures, but may solely reflect the concentration of cytoplasmic constituents. It is difficult to differentiate between these possibilities on account of the variability of dendritic and axonal size and number of tubules, but there is no positive evidence at any level in favour of such a generation, such as sprouting, branching or division of tubes.

Transneuronal degeneration appears to occur in only certain cells in the central nervous system, particularly in the sensory pathways (see Matthews et al. 1960) and may represent a sensitivity of neurons with particular properties to a fall in the level of this afferent, presumably excitatory, input. For this reason it is necessary to examine the different synaptic properties of the cells of the olfactory system that undergo transneuronal change, with a view to finding a common factor that is changed in all cases. If the correlation of vesicle shape and type of membrane thickening with functional activity holds true for the olfactory bulb (see Rall et al. 1966; Price & Powell, 1970b), the granule cells and periglomerular cells (Pinching, 1970) may be considered as inhibitory neurons. The main excitatory input to the mitral and tufted cells, therefore, is that from the olfactory nerve terminals in the glomeruli. For periglomerular cells, the olfactory axons are also the major excitatory input, but other excitatory influences are contributed by the axon collaterals of the tufted cells and by centrifugal fibres (Pinching, 1970). Intrinsic axons, including these collaterals, may be affected only secondarily and may not necessarily undergo transneuronal degeneration, and centrifugal fibres are not affected at all. The excitatory influences on to granule cells include the axon collaterals and the dendrites of mitral and tufted cells, as well as terminals of centrifugal axons and those from the anterior olfactory nucleus and the anterior commissure (Price & Powell, 1970c). Short-axon cells, both of the deep layers and the glomerular layer, have occasionally been seen to show degenerative changes at long survival periods; these cells receive a collateral input from mitral and tufted cells, together with other intrinsic and extrinsic (excluding olfactory) inputs. The pyriform cortex receives the axon terminals of mitral and tufted cells in the superficial third of the plexiform layer, but also receives other afferent inputs.

Thus of the cells of the olfactory system that show transneuronal changes, as seen with the electron microscope and with the Golgi technique, the mitral, tufted and periglomerular cells are directly deprived of the whole or a large part of their afferent (excitatory) input, although they retain other, mainly inhibitory, inputs; all the other cells affected, which have no primary contact from the olfactory nerves, have a common input from the axons, collateral or terminal, of the mitral and tufted cells, which have themselves been to a large extent deafferentated. It may be seen, therefore, that although normal boutons may persist on affected cells, and in some cell types none is subject to orthograde degeneration due to experimental interruption, the balance of excitatory and inhibitory inputs has been altered in all cases, so that they may be unable or only rarely able to reach the threshold for producing a propag-
gated impulse. This poverty of depolarizing input or spike output could give rise to an alteration in cellular activity resulting in the morphological changes. The simplest explanation, therefore, is that the cellular change has an ionic basis, in which the movement of ions involved in the depolarization of the membrane and its subsequent recovery is essential to the normal fluid and ion balance between the cell and its surroundings. The altered appearance of the extracellular and glial space consistently associated with the glomerular arborizations of the degenerating cells may reflect the simultaneous alteration in the composition of the extracellular space and glial cytoplasm; the neurons showing a watery cytoplasm may also be related to this process. Alternatively, the reduction of spike generation at the initial segment may cause primary changes at the level of the cell soma, which are then reflected throughout the whole of the cellular ramifications, the axons being the last to be affected, although this mechanism cannot explain the changes in dendrites and spines of the pyriform cortex.

Whatever its cellular basis, the 'orthograde' transneuronal degeneration of cells across more than one synapse (see Powell & Erulkar, 1962) represents a form of functional deafferentation in which the number of excitatory impulses is reduced in a given pathway, without any primary alteration in inhibitory or other excitatory inputs. The effects of functional deafferentation, without any neuron injury, have been described in the visual pathway of the kitten (Wiesel & Hubel, 1963a, b; Hubel & Wiesel, 1970) and the mouse (Valverde, 1967), and the former authors have noted cellular changes in the lateral geniculate nucleus at the light-microscopic level similar to those occurring in transneuronal degeneration of that nucleus after eye removal; Valverde has noted the loss of spines in the visual cortex, as seen with the Golgi method, and Coleman & Riesen (1968) have demonstrated a reduction in the number of stellate cell dendrites and in their length. It would be of interest to determine whether electron-microscopic changes similar to those occurring in the olfactory system after olfactory nerve section can be found in the neurons of the geniculocortical pathway in conditions of light deprivation. Wiesel & Hubel (1963a, b) have also shown that the geniculate neurons may be excited in the normal way, although their physiological effects at cortical level have been affected; this is consistent with the finding that the cells of the olfactory pathway maintain a level of cytological organization, and may be considered capable of normal activity. This has been confirmed by the physiological study of the transneuronally degenerating olfactory bulb of the rabbit (C. G. Phillips, T. P. S. Powell & G. M. Shepherd, unpublished observations of 1960) in which the responses of the mitral cells to antidromic stimulation of the lateral olfactory tract were studied. The evoked potentials following the tract shock were found to be of normal pattern and of normal or only slightly reduced amplitude; the correlation of wave pattern with histological depth was also normal. The conclusion could therefore be drawn that the mitral cells generate antidromic impulses in the normal manner and the mitral-to-granule excitatory synapses also operate normally. The findings also suggest that the granule-to-mitral inhibitory synapses are also capable of operating normally.

If the whole-cell transneuronal degeneration in the olfactory bulb and lateral geniculate body, and the more limited dendritic degeneration in the pyriform and
Transneuronal cell degeneration in the olfactory system

striate cortices may be considered analogous, the present observations could throw some light on the anatomical basis for the physiological alterations in cortical function described by Wiesel & Hubel (1963b). These authors note that the ‘abnormality is in the region of the synapse between the axon terminals of geniculate cells (those receiving input from the deprived eye) and the cortical cells on which these terminals end’. In view of the fact that transneuronal degeneration of axons in the olfactory system is exactly comparable to the degeneration of their parent cell somata and dendrites, it could reasonably be considered that these axons too were excitable in the normal way, the ‘degeneration’ representing an altered functional state rather than cell death. On the other hand, the selective transneuronal degeneration of some spines and dendrites of the pyriform cortical neurons without these changes necessarily occurring in the cell somata and other dendrites, may cause the degenerating components to lose their functional importance in the spike generation. Thus, although the altered dendrites and spines may be responsive to depolarizing input, the other normal dendrites could have become ‘dominant’ in determining the cell’s responses, due to a functional adaptation in the neuron to the altered dendritic input.

It has been noted that transneuronal changes occur at different rates in different species (Matthews et al. 1960). The same has been found in the olfactory system with both electron-microscope and Golgi techniques: the rabbit shows cellular changes at 10 days after operation, while no changes are found in the rat with either technique until 200 days, suggesting that the rat is better able to maintain neuronal structure, and therefore presumably function, than the rabbit. Differences in the time course of transneuronal degeneration between different species have been observed in the visual system (Cook et al. 1951; Matthews et al. 1960). No obvious differences have been found in the neuronal organization of the olfactory bulbs of the two species at electron-microscopic level, nor in the time course of the orthograde degeneration of the olfactory nerves; there is no evidence of reinnervation in either rat or rabbit. It should be noted that both species have been operated on at an immature age (5–7 weeks) and that this may be a critical factor in determining the severity or even the occurrence of a degenerative alteration (Matthews et al. 1960; Hubel & Wiesel, 1970).

It is a striking fact that in both rat and rabbit the onset of transneuronal cell change corresponds with a distinct decrease in the number of post-synaptic thickenings without presynaptic terminals, and of fine terminal parts of dendrites. Clearly it is not possible with these methods to determine which factor is the primary cause of the onset of cellular change, but it could be suggested that the presence of post-synaptic thickenings until the appearance of transneuronal change in the rat is directly related to the persistence of the fine dendritic terminals in the glomeruli of this animal and the absence of transneuronal change; this could be considered as a transient manifestation of denervation hypersensitivity. The loss of spontaneous activity in the region of the post-synaptic thickenings could be responsible for terminating the prevention of cellular degeneration by these elements.
Golgi studies

Experimental Golgi studies have shown that cells or parts of cells that have been subject to deafferentation do not impregnate with this technique or are resistant to it (Matthews & Powell, 1962; Jones & Thomas, 1962; White & Westrum, 1964; Globus & Scheibel, 1966; Valverde, 1967; Powell, 1967; Coleman & Riesen, 1968). The correspondence in time course between the ultrastructural changes and the failure of Golgi impregnation in the olfactory system undergoing transneuronal degeneration is significant in this regard. It should be noted that while the degenerative changes occur throughout the affected cells of the olfactory bulb, the changes in the pyriform cortex affect mainly dendrites and/or spines, rather than whole cells; the latter observation has also been made in the visual cortex (Globus & Scheibel, 1966; Valverde, 1967; Coleman & Riesen, 1968), but reasons for the difference are obscure.

Two related explanations may be put forward in relation to the failure of Golgi impregnation: (1) The traditional view of the Golgi technique has been recently restated (Schapiro & Vukovich, 1970); that is, that it stains only those neurons that are chemically, and therefore functionally, different at the time of fixation (i.e. those that have been active just before fixation). If transneuronally degenerating cells are inexcitable due to an insufficient number of functional excitatory synapses, they may never reach the chemical state necessary for impregnation. (2) The Golgi technique has been shown to be a cytoplasmic stain, by combined Golgi-electron microscopic studies (Blackstad, 1965; Stell, 1967; A. J. Pinching, unpublished observations), and spares the membrane-bound systems. If the reaction is with the 'free' cytoplasmic component, the reduction of this in transneuronally degenerating cells could reduce the likelihood of their being impregnated.

The characteristic impregnation of the 'altered mitral cell' in transneuronal material, that is, the mitral cell and the granule cells with peripheral processes adjacent to the mitral cell body, bears on this problem. If the impregnation of these granule cells represents their functional activity, they would be acting to inhibit the mitral cell (Rail et al. 1966; Price & Powell, 1970b), yet this latter cell does stain. Alternatively, they could be considered as 'trying' to excite the mitral cell, by a reduced activity which may also make them chemically different, the staining of the mitral cell, in spite of its cytoplasmic poverty, indicating the success of the attempt. A further explanation could be that the mitral cells are able to give rise to an impulse spontaneously and, in doing so, excite the granule cells via the dendro-dendritic synapses on to their peripheral processes; the constant relationship of the impregnated granule cells to the soma of the altered mitral cell could be taken as suggesting a somatic rather than dendritic origin for the postulated impulse which affects primarily those granule cells whose gemmules synapse with the mitral cell soma. This anomalous finding on the altered mitral cells of transneuronal degeneration and their attendant granule cells points clearly to the inadequacy of our knowledge of the mechanism of the Golgi method, though it indicates that functional rather than simply morphological interpretations of experimental material using this method may be necessary.

The cases of dendrites or their spines failing to impregnate while their parent
Transneuronal cell degeneration in the olfactory system

processes remain normally stained (Jones & Thomas, 1962; White & Westrum, 1964; Globus & Scheibel, 1966; Valverde, 1967; Coleman & Riesen, 1968) are still open to several explanations, although the phenomenon is beyond doubt. Either the spine or dendrite undergoes a change similar to transneuronal degeneration without the same changes occurring in the parent process, and the Golgi stain fails on this account, whether it is functional or morphological or both; or the spine or dendrite is lost entirely, as has been considered by most observers (Globus & Scheibel, 1966; Mouren-Mathieu & Colonnier, 1969). The question of the age of the animal at operation is clearly critical in differentiating between failure of spine development and actual spine loss. To try to differentiate between these possibilities is not advisable on the present evidence, although the observation of degeneration of spines which receive synapses from normal terminals in this material indicates that the transneuronal changes in them cannot simply be ascribed to the degeneration of individual axon terminals.

The clear correspondence between Golgi and electron-microscopic observations on the olfactory systems of rabbit and rat, both in the time course and the nature of the cellular changes of transneuronal degeneration, shows the complementary nature of these very different techniques. The fact that transneuronal degeneration can be observed as a clearly defined phenomenon in both, each demonstrating quite distinct characteristics in comparison with normal material, is strong evidence for the validity of both sets of findings.

The results of the studies on transneuronal cell degeneration with 3 techniques—thionin and Golgi for light microscopy, and electron microscopy—require further correlation in respect of the proportion of cells of any type that are affected. The thionin results indicate a decrease in size for all cells of any type, although the ranges of normal and degenerate cell areas do overlap, suggesting that some cells could be entirely unaffected; Golgi-Cox impregnation, however, shows that all but the few ‘altered’ mitral and tufted cells are resistant to impregnation after denervation. Approximately one third of any type appears unaffected at the electron-microscopic level, although they may have shrunk slightly without obvious qualitative change. The fact that most of the ‘altered’ mitral and tufted cells of Golgi preparations have atypical shapes and appear very shrunken suggests that those cells which are resistant to Golgi impregnation after deafferentation include the cells that seem least affected at the electron-microscopic level. Thus the subtlest transneuronal changes after deafferentation appear to be reflected most sensitively by the failure of Golgi impregnation; this in turn favours the suggestion that this failure is primarily due to a functional change, rather than a morphological one.

Transneuronal and spontaneous degeneration

The occasional presence of similar dark degenerating neurons in other parts of the brain, notably in sensory systems, may be considered in relation to these findings as representing the spontaneous degeneration of these neurons due to a reduction or loss of activity of peripheral or central origin. On the other hand, transneuronal degeneration could be regarded as an increased level of spontaneous degeneration brought on
by deafferentation, either functional or pathological. The occurrence of 'dark profiles in apparently-normal central nervous system' (Cohen & Pappas, 1969), which may well have such an origin as discussed above, need not bring into question the application of experimental degeneration studies, although it may make these more difficult, for two main reasons: first, on account of the clear distinction between the types of change taking place in orthograde and transneuronal axon terminal degeneration, which are morphologically similar at only the early stages of the former; and secondly, these spontaneous degenerative changes are rare in comparison with the terminal degeneration produced by the section of an afferent pathway, so that even if they are confused morphologically, they should not affect the large sampling of degenerating terminals that is essential for the electron-microscopic identification of the mode of termination of that afferent pathway. The use of young animals to avoid a high incidence of spontaneous degeneration and the study of orthograde degeneration at several survival periods, to demonstrate a sequence of terminal changes, should distinguish clearly between it and the spontaneous degenerative changes similar to those seen in transneuronal degeneration of axon terminals. Careful examination of the normal side and direct comparison of the two sides in each animal is obviously essential.

Experimental value of transneuronal degeneration

The occurrence of transneuronal degeneration has been used with the light microscope to determine the organization of the projections of certain pathways, for example the retinal projection to the lateral geniculate body (Le Gros Clark & Penman, 1934). Similarly, cellular alterations of the kind described above are of great value as markers in the electron-microscopic analysis of a part of the central nervous system: to be able to identify a specific cell or cell type throughout its extent without requiring continuity in a single or a limited number of planes for identification, greatly facilitates the predominantly 2-dimensional approach that is inevitable with the use of the electron microscope. A similar suggestion has been made by Grant & Westman (1968, 1969) in regard to the cellular changes occurring in retrograde degeneration. Clearly the question of cell specificity of the transneuronal change must be carefully considered in any region, but together with other criteria, normal and experimental, as well as Golgi evidence, this type of marking could be of considerable importance, particularly for the identification of intrinsic connexions. In the olfactory bulb, for instance, this approach has considerably clarified the problem of the mode of termination of the recurrent collaterals of the mitral and tufted cells.

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Transneuronal cell degeneration in the olfactory system


272

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**ABBREVIATIONS ON PLATES**

| c | normal cell soma |
| cy | cytoplasm |
| d | normal dendrite |
| da | transneuronally degenerating axon |
| dc | transneuronally degenerating cell soma |
| dd | transneuronally degenerating dendrite |
| dg | transneuronally degenerating gemmule |
| dp | transneuronally degenerating granule |
| ds | transneuronally degenerating spine |
| dt | transneuronally degenerating terminal |
| e | extracellular space |
| er | endoplasmic reticulum |
| fi | fibrillar portion of nucleolus |
| g | normal gemmule |
| gl | glia |
| go | Golgi apparatus |
| gr | granular portion of nucleolus |
| is | initial segment of axon |
| ly | lysosome |
| m | mitochondrion |
| my | myelin sheath |
| n | nucleus |
| no | nucleolus |
| ot | orthograde degeneration of terminal |
| p | pedicle |
| r | rodlet |
| s | normal spine |
| t | normal terminal |
| v | vacuole |
| z | nuclear indentation |

Arrow-heads indicate synaptic specializations from the post-synaptic side. Double arrow-heads indicate persisting post-synaptic membrane thickenings.

All illustrations are from rabbit olfactory bulb unless stated otherwise.

Fig. 1. Normal mitral cell with beginning of primary dendrite. × 7,400.

Fig. 2. Transneuronal degeneration of primary dendrite of mitral cell in Fig. 3, passing between predominantly normal processes in deep part of external plexiform layer. 70-day survival. × 6,200.
Transneuronal cell degeneration in the olfactory system
Fig. 3. Moderate transneuronal degeneration of mitral cell, with beginning of primary dendrite. 70-day survival. (Note the lower magnification than in Fig. 1.) × 6200.

Fig. 4. Mild degeneration of mitral cell. 70-day survival. × 7000.
Transneuronal cell degeneration in the olfactory system
Fig. 5. Mild degeneration of granule cell with peripheral process and gemmule receiving synapse. 70-day survival. $\times 9200$.
Fig. 6. Severe degeneration of granule cell with somatic spine (see Fig. 10). Note part of normal granule cell (right) and mild degenerate granule cell (left). 49-day survival. $\times 15700$. 
Transneuronal cell degeneration in the olfactory system
Fig. 7. Detail of moderate degeneration in a mitral cell, showing the characteristics of nucleus and cytoplasm. Note the intact pores in the nuclear membrane around the cytoplasmic indentation (arrows). 90-day survival. $\times 39,000$.

Fig. 8. Detail of an intranuclear fibrillar rodlet from a degenerating tufted cell. 49-day survival. $\times 54,900$.

Fig. 9. Detail of the nucleolus in severe degeneration of a mitral cell, showing segregation of nucleolar components (cf. Fig. 1). 49-day survival. $\times 26,000$.

Fig. 10. Detail of somatic spine of the granule cell in Fig. 6, receiving a synapse from an apparently normal axon terminal. 49-day survival. $\times 35,600$. 

A. J. Pinching and T. P. S. Powell
Transneuronal cell degeneration in the olfactory system
Fig. 11. Initial segment of tufted cell axon in the external plexiform layer, receiving a symmetrical synapse, possibly itself undergoing mild transneuronal degeneration. Note limited tubular aggregation (arrow) and absence of plasma membrane under-coating. 90-day survival. × 35,000.

Fig. 12. Degenerating mitral cell dendrite in external plexiform layer entering into a reciprocal synapse with the normal gemmule of a granule cell. 70-day survival. × 41,000.

Fig. 13. Orthograde degeneration of olfactory nerve terminals in glomeruli with synapses on to a transneuronally degenerating dendrite and a normal dendrite. 10-day survival. × 30,000.

Fig. 14. A degenerating dendrite and a normal dendrite in glomerulus showing 3 exposed post-synaptic membrane thickenings after loss of olfactory nerve terminals. The 2 apposed thickenings show 2 layers of extracellular material in an unusually wide cleft. 18-day survival. × 66,900.
Fig. 15. A normal gemmule in the external plexiform layer entering into a reciprocal synapse with a degenerating mitral cell dendrite and receiving an asymmetrical synapse from a degenerating recurrent collateral. Synaptic polarities and axon degeneration were confirmed by serial sections. 70-day survival. × 24,700.

Fig. 16. A degenerating dendrite in the external plexiform layer. Rat. 200-day survival. × 33,000.

Fig. 17. Fine degenerating terminal portions of dendrites in glomerulus, surrounded by marked extracellular space. 18-day survival. × 46,300.

Fig. 18. Degenerating varicose terminal portion of mitral or tufted cell dendrite in glomerulus, with marked extracellular space. 70-day survival. × 36,000.
Transneuronal cell degeneration in the olfactory system
Fig. 19. Transverse section of secondary dendrite of mitral cell in moderate degeneration showing cross-section of neurotubules. 70-day survival. × 92,200.

Fig. 20. Transverse section of secondary dendrite of mitral cell from the normal side of the brain used for Fig. 19. × 92,200.

Fig. 21. Dark degenerating gemmule with reciprocal synapse in glomerular layer. 10-day survival. × 44,000.

Fig. 22. Pale degenerating gemmule with reciprocal synapse and attenuated pedicle in the glomerular layer. 24-day survival. × 32,600.

Fig. 23. Degenerating dendrite in the pyriform cortex with apparently normal asymmetrical and symmetrical synapses. 70-day survival. × 53,200.
Transneuronal cell degeneration in the olfactory system
Fig. 24. Degenerating myelinated fibre in pyriform cortex. 33-day survival. × 37800.

Fig. 25. Degenerating axon terminal of tufted cell recurrent collateral terminating on to a degenerating periglomerular cell spine in the glomerular layer. 18-day survival. × 30800.

Fig. 26. Degenerating terminal of mitral or tufted cell collateral in the granule cell layer. 49-day survival. × 37600.

Fig. 27. Degenerating spine and dendrite in pyriform cortex with apparently normal terminal synapsing on to it. Note glial engulfment. 70-day survival. × 45400.

Fig. 28. Degenerating axon terminal in pyriform cortex. 33-day survival. × 29800.

Fig. 29. Normal terminal, spine and dendrite in pyriform cortex. × 24900.
Transneuronal cell degeneration in the olfactory system