AN ELECTRON-MICROSCOPIC STUDY OF
THE TERMINATION OF THE AFFERENT
FIBRES TO THE OLFACTORY BULB FROM
THE CEREBRAL HEMISPHERE

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SUMMARY
An experimental investigation has been made of the site and mode of termination of the
3 groups of afferent fibres to the olfactory bulb which come from more caudal parts of the
cerebral hemisphere. Lesions have been placed in the relevant parts of the brain of the rat and
the resulting degeneration of axon terminals in the olfactory bulb studied with the electron
microscope. All 3 groups of these extrinsic afferent fibres end in asymmetrical synapses upon
the granule cells, and they have a differential termination upon its various processes. The possi-
ability that these fibres also end upon other cells in the bulb (particularly the short-axon and
periglomerular cells) cannot be excluded.
The centrifugal fibres end upon gemmules in the deep half of the external plexiform layer
only; no degenerating terminals were found in relation to the glomeruli although degenerating
centrifugal axons are present here. The fibres of the anterior commissure terminate upon spines
and varicosities of the deep dendrites and upon somatic spines of the granule cells. After lesions
of the anterior olfactory nucleus, degenerating terminals were found in the ipsilateral olfactory
bulb, which could not be ascribed to the centrifugal fibres or to the fibres of the anterior
commissure, as they ended upon the spines of peripheral processes in the granule cell layer, and
upon gemmules in the superficial as well as in the deep half of the external plexiform layer.
It is proposed that these terminals are those of the axon collaterals from the ipsilateral
anterior olfactory nucleus. The axons which form symmetrical synapses, and many which form
asymmetrical synapses, do not degenerate even after a lesion immediately behind the olfactory
bulb, and are therefore intrinsic to the bulb. It is suggested that the axons which are associated
with symmetrical synapses are those of the short-axon cells, and the asymmetrical synapses are
formed by the axon collaterals of the mitral and tufted cells.

INTRODUCTION
The afferent fibre connexions of the olfactory bulb have been extensively studied
with the light microscope. In addition to the olfactory nerves which come from the
nasal mucosa, and which end superficially in the glomeruli of the bulb, there are 3
different fibre pathways to the bulb from more caudal parts of the cerebral hemisphere
and the origin and course of these forwardly directed fibres have now been determined.
The fibres which form the anterior commissure are fine and arise in the anterior
olfactory nucleus of the contralateral side (Lohman, 1963), while the coarser centri-
fugal fibres have their origin in the nucleus of the horizontal limb of the diagonal
band and pass forwards in close association with the main efferent tract of the bulb,
the lateral olfactory tract (Price, 1969a; Price & Powell, 1970a). The third group of
these afferent connexions are the collaterals of the axons which enter the anterior
commissure from the anterior olfactory nucleus, and which turn rostrally to enter the 
ipsilateral olfactory bulb along with the commissural fibres from the opposite side 
(Valverde, 1965). Because of the differences in origin and course between the fibres of 
the anterior commissure and the centrifugal fibres it is possible to interrupt these 
selectively, but as both these groups of fibres traverse the anterior olfactory nucleus 
before they enter the bulb it is not possible to damage selectively the axon collaterals 
from this nucleus.

These neurohistological investigations have also provided evidence that the different 
groups of afferent fibres may terminate at different levels in the olfactory bulb, the 
fibres of the anterior commissure being confined to the granule cell layer, while the 
centrifugal fibres reach as far superficially as the glomerular layer. However, with 
light-microscopical techniques it has not been possible to define the precise level of 
termination of these fibres, because the degeneration which is found in a given layer 
may be that of fibres of passage only, and not of axon terminals; in the olfactory bulb 
there is the additional difficulty of obtaining unequivocal staining of terminal de-
generation with silver methods: the precise mode and site of termination of these 
fibres in relation to a specific cell, or part of a cell, cannot be determined with the 
light microscope.

Electron-microscopic studies of normal material of the olfactory bulb have raised 
further questions, for they have shown that several different types of pre- and post-
synaptic processes can be identified (Andres, 1965; Price & Powell, 1970b, c). By 
itself, however, an investigation of normal material does not readily permit the identi-
fication of all these processes, especially those which arise outside the bulb.

In order to obtain a more complete understanding of the neuronal connexions of 
the olfactory bulb, or of any other part of the brain, therefore, it is necessary to apply 
the techniques of electron microscopy to experimental material. The results of such 
an experimental investigation, in which an attempt has been made to define the site 
and mode of termination of those afferent fibres to the olfactory bulb which come from 
more caudal parts of the cerebral hemisphere, form the basis of the present paper; 
after lesions placed in the relevant parts of the brain the terminal degeneration in the 
olfactory bulb has been studied with the electron microscope (Price, 1969b).

MATERIALS AND METHODS

Lesions were placed in the brain of 47 rats, under intraperitoneal Nembutal anaesthesia. 
In 31 of these, the lateral olfactory tract was approached through the temporal region, and the 
tract and the adjacent cortex were damaged under direct vision; of this group 19 lesions were 
restricted to the lateral olfactory tract, while in the remaining 12 animals the anterior olfactory 
nucleus and the anterior limb of the anterior commissure were also involved. In 16 brains, the 
olfactory bulb and most of the anterior olfactory nucleus of one side were removed by suction 
through a hole in the dorsal part of the skull. After survival periods of 2½–6 days, the animals 
were perfused and the olfactory bulbs prepared for electron-microscopic examination in the 
manner described previously (Price & Powell, 1970b). The more caudal parts of the brains 
were dehydrated and embedded in paraffin; serial sections were taken over the extent of the 
lesions, and stained with thionine to determine the precise site and extent of the damage. It 
should be emphasised that this procedure was absolutely essential in order to know exactly which 
structures had been involved in each experiment.
The distribution of degenerating terminals within the olfactory bulb after the various lesions was determined first by reference to the morphological features of the different layers of the bulb, and also more precisely by taking micrometer measurements with the electron microscope of the position of each degenerating terminal, according to the method described by Alksne, Blackstad, Walberg & White (1966). To make these 'maps' of the distribution of degenerating terminals 2 types of section were used; the first of these was up to 1 mm\(^2\) in size in order to span one or more whole layers; the second type was long and thin, approximately 750 \(\mu m\) by 90 \(\mu m\), cut with the knife edge parallel to the long axis (Sjöstrand, 1967), and it was possible to include several layers of the bulb in these sections, allowing precise orientation and mapping, without sacrificing the sectioning quality characteristic of small sections. Both types of section were mounted on single-hole grids coated with Formvar, usually 1 of the larger type of section per grid and 20–30 of the narrow type. For the identification of the postsynaptic profiles contacted by degenerating terminals, the use of serial sections proved invaluable; ribbons of between 20 and 150 serial sections (either the long narrow type or smaller ones of approximately 100 \(\mu m^2\)) were mounted on Formvar-coated single-hole grids.

**RESULTS**

*Mode of degeneration and appearance of degenerating profiles*

After all 3 types of lesion used in these experiments the terminal degeneration found within the olfactory bulb consisted of shrunken, electron-dense profiles, which often contain distorted mitochondria and vesicles; no example of the type of degeneration characterized by a proliferation of neurofilaments and swelling of the axon terminal has been found. At the shorter survival periods degenerating terminals commonly showed only a general darkening of their cytoplasm, with little or no distortion of their internal or external features; their shape was still relatively normal, and the vesicles and mitochondria within them did not appear to have been altered (Fig. 8). There was usually no evidence of a glial reaction at this stage. At a slightly later stage, however, the degenerating terminals appeared to be compressed by the surrounding processes, and the vesicles were clumped together and distorted; the cytoplasm was then very much darker, or in some examples the ending was filled completely with vesicles, so that the vesicles stood out as pale circles against a dark background (Fig. 26). The mitochondria were swollen, with distorted cristae, and often filled most of the terminal; these mitochondria have been aptly described as having a 'glassy' appearance. Although the cytoplasm had usually become quite electron-dense by this stage (Figs. 18, 19), this was not always so, and in some cases the terminal still appeared relatively light (Fig. 9). By this time the gial reaction had also become obvious, and tongues of astroglia could be seen invading and fragmenting the terminal. Frequently the compression of the degenerating terminal on each side by glial processes resulted in the portion of the terminal away from the membrane thickening becoming considerably attenuated to form a long, curved tail (Fig. 10). These reactive glial processes usually had a 'granular' cytoplasm which contained many more glycogen granules and other inclusions than the glia found in normal material. In the later stages degenerating terminals became still more shrunken, and appeared very dark; most of the terminal had usually been ingested by the surrounding glia, leaving a relatively thin crescent of the degenerating profile near the synaptic region (Fig. 11).
Especially in the later stages of degeneration, the only valid criterion for the identification of a degenerating terminal is the presence of a synaptic membrane thickening. In all 3 fibre systems which have been investigated in the present study the synaptic thickening was always found to be of the asymmetrical type. At all stages of degeneration the thickening of the postsynaptic membrane, together with the electron-dense material adherent to its cytoplasmic side, have always been found to be unaltered.

As was suggested by Westrum (1966) from a study of degeneration in the pyriform cortex, however, the degenerating terminal and the thickened presynaptic membrane sometimes appeared to be displaced from the synaptic region by glial processes, which then occupied the space immediately opposite the postsynaptic thickening (Fig. 12). This isolation of the postsynaptic thickening seemed in some cases to have occurred by retraction of the degenerating terminal, and in such cases the ‘presynaptic’ position was reoccupied by neuronal profiles (Figs. 13, 14). No thickening of the opposed part of the membrane of the reoccupying profile, or any other indication of a new synapse being formed, has ever been seen, but only a few examples of this relationship have been found, and these have not been studied in serial sections. Whether these findings may indicate the early stages of re-innervation must therefore remain uncertain, but they should be investigated further in experiments with longer survival periods.

The degenerative changes which occurred in myelinated and unmyelinated axons were very similar to those which have been described above for terminal degeneration. The most characteristic and consistent changes were an increased density of the axoplasm, distortion of mitochondria, and, at a later stage, vacuolation of the axoplasm (Fig. 17). The myelin sheath usually appeared unchanged in the earlier stages of degeneration, but later it may be reduplicated, and/or distorted into weird shapes. In longitudinal section degenerating myelinated axons often have localized swellings along their length. Fig. 15 is an example of such a swelling on an axon which showed a more unusual form of axonal degeneration; there was an accumulation of mitochondria and of dense bodies, and an apparent disruption of the neurotubules. The axoplasm in the centre of the fibre remained relatively light; it is abnormally flocculent and filamentous in appearance.

In the later stages of degeneration, changes were also found in the postsynaptic profiles. These profiles often surround the degenerating terminal on at least 3 sides, as if the postsynaptic process were expanding to fill the space left by the shrinkage of the degenerating terminal (Figs. 9, 22). In serial sections, however, no degenerating presynaptic profile was ever found to be completely enclosed by a neuronal profile. In many examples the postsynaptic profiles appeared to be shrunken and distorted, and enclosed by glia, although serial sections have never shown a postsynaptic profile completely separated from its parent cell.

The centrifugal fibres to the olfactory bulb

The termination of the rostrally directed fibres in the lateral olfactory tract which arise in the ipsilateral nucleus of the horizontal limb of the diagonal band (Price, 1969a) has been investigated in those brains in which the lesion was strictly confined
to the lateral olfactory tract and the adjacent pyriform cortex and olfactory tubercle. It should be emphasized that the site and extent of all lesions were determined histologically, and that any brain in which the lesion encroached upon the anterior commissure or the anterior olfactory nucleus was not included in this group; such brains will be described in the next two sections.

Fig. 1. Composite map of the distribution of degenerating terminals of centrifugal axons after a lesion of the lateral olfactory tract. It can be seen that the degenerating terminals (×) are concentrated in the deep half of the external plexiform layer.

The maximum density of degenerating terminals of centrifugal fibres was found at a survival period of 5 days, and most of the observations reported here were made on such material. With a survival period of 3 days, degenerating terminals were very sparse, and almost exclusively of the type described above as early degeneration, so that they were difficult to distinguish from relatively dark, but probably normal terminals. After a survival period of 5 days most of the degeneration corresponds to the middle-to-late stages, and could be identified with certainty; at 6 days, the degeneration was little different from 5 days, except that it was again slightly more advanced.

With a lesion restricted to the lateral olfactory tract, degenerating axons were present in all layers of the olfactory bulb, but degenerating terminals have been found only in the deeper half of the external plexiform layer; this is best shown by the micrometer ‘mapping’ technique (Alksne et al. 1966), with which the precise position of each terminal can be determined (Fig. 1). Although complete ‘maps’ of this sort were compiled from material from only 3 animals, all of which had survived for 5 days after the operation, qualitative observations on all the other brains which were examined were in accord with these findings. Degenerating axons were very plentiful in the granule cell layer, and also among the periglomerular cells superficial to the external plexiform layer; this is the distribution which would be expected from the light-microscopic
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studies with the Nauta method. Although the degenerating axons in the granule cell layer are presumably fibres passing through the deeper layers of the bulb to reach the external plexiform layer, the degenerating axons in the periglomerular layer cannot be explained in this way. Even after intensive examination of this region in brains with survival periods of 2, 4, 5 and 6 days, no unequivocal degenerating terminals have been found, and the question of the termination of these superficially lying fibres remains unsolved.

Fig. 2. Drawings based on wax reconstructions of serial sections to show degenerating terminals of centrifugal fibres ending on gemmules of granule cells. (c, centrifugal fibre; g, gemmule; m, mitral cell dendrite; p, peripheral process of granule cell, r, reciprocal synapse.)

The degenerating axon terminals in the external plexiform layer have been found to form exclusively asymmetrical synaptic contacts with profiles which contain a high concentration of relatively large, flattened vesicles (Figs. 9, 10). From the investigations on normal material (Price & Powell, 1970b), it is known that the only processes in the external plexiform layer which correspond with this description are the gemmules attached to the distal portions of the peripheral processes of the granule cells. In order to identify these processes with certainty, however, it was considered necessary to find a degenerating terminal upon a vesicle-containing profile which was connected to a peripheral process in a spine-like manner, and which participated in a reciprocal synapse with a mitral or tufted cell dendrite. Although examples were found in single sections of degenerating terminals which ended upon gemmules attached to peripheral processes, in order to satisfy both criteria serial sections were required. An example of such a series is shown in Fig. 19, and a drawing based on a wax model of these same sections is reproduced in Fig. 2 (Price, 1968). It can be seen that two gemmules arise from the same peripheral process, one of which is postsynaptic to a
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degenerating terminal of a centrifugal axon, while the other forms a reciprocal synapse with a mitral or tufted cell dendrite. Another drawing based on a similar series is shown in Fig. 2; in this case a single gemmule receives a degenerating centrifugal terminal and participates in a reciprocal synapse with a mitral or tufted cell dendrite. It can therefore be concluded that the centrifugal fibres to the olfactory bulb end in the deep half of the external plexiform layer upon the gemmules on the distal portions of the granule cell peripheral processes. That these centrifugal fibres can form synapses with several gemmules is indicated by two distinct observations. First, the degenerating terminals of these fibres have frequently been found to make synaptic contact with several gemmules (Fig. 19), and secondly, degenerating fibres have been found in serial sections which form several boutons en passant, each of which may synapse with more than one gemmule (Fig. 16).

Although many degenerating terminals of centrifugal fibres were found upon gemmules which participated in reciprocal synapses, occasionally a degenerating terminal was found upon a process which contained relatively large, flattened vesicles and was attached to a peripheral process of a granule cell, but which did not appear to be related to a reciprocal synapse, even when studied throughout its extent in serial sections. It is possible that there were reciprocal synapses off such processes which lay precisely parallel to the plane of section, so that they would be obscured by the passage of the knife, but as such appendages were encountered in several series this explanation would seem improbable. The identity of these appendages has not been determined, but they might be rare examples of spines arising from the peripheral processes in the external plexiform layer.

The fibres of the anterior commissure

The fibres from the contralateral anterior olfactory nucleus which cross in the anterior limb of the anterior commissure to terminate in the olfactory bulb have been studied in those brains in which the olfactory bulb and anterior olfactory nucleus of one side had been destroyed, or in which a lesion of the lateral olfactory tract had also encroached upon the anterior olfactory nucleus and the anterior commissure. In the olfactory bulbs contralateral to such lesions degenerating axons and terminals were found in the granule cell layer and, very rarely, in the external plexiform layer.

For the experimental study of this projection the optimum survival time appears to be 3 days, or possibly even less. Even at 3 days degenerating terminals are relatively sparse, and by 5 days they have almost entirely disappeared; in some brains with the longer survival period none of these terminals could be found at all. Most of the observations on the termination of the anterior commissure were therefore made on animals which had survived for 3 days after the operation.

A 'map' of the position of degenerating terminals after a lesion of the anterior commissure is shown in Fig. 3. These degenerating terminals are found throughout the granule cell layer; although none was found in relation to the dendrites deep to the deepest granule cell bodies, several were found at the level of these cells. It may be noted from Fig. 3 that the density of degeneration in these experiments is less than that found after the other types of lesions which have been studied. An additional
point about the distribution of the terminals of the fibres of the anterior commissure is that they tend to be clustered into small groups of two or more (Fig. 20); this clustering is especially obvious in serial sections, where, over several sections, 4 or 5 degenerating terminals could often be found in the immediate vicinity of each other.

Most of the postsynaptic profiles upon which the degenerating terminals of the fibres of the anterior commissure end can be identified as spines, because they have a grey, flocculent cytoplasm, lack neurotubules, and frequently contain a spine apparatus. The majority of these spines also contain relatively large, flattened vesicles of the type which have been shown to be present in the spines of the granule cells (Price & Powell, 1970c). By the use of serial sections the spines which receive these degenerating terminals have been found to arise from the cell somata of granule cells (Fig. 21),

![Diagram](image)

Fig. 3. Composite map of the distribution of degenerating axon terminals (x) after a lesion of the anterior commissure. There are relatively few terminals, all of which are situated in the granule cell layer.

from medium to small sized dendrites (Fig. 24), and from varicose dendrites (Fig. 26); the last of these could be identified by the sparseness of the neurotubules in the swollen portions, and by the fact that these portions tapered off to much smaller dendritic shafts at both ends. With one possible exception, no degenerating terminal has ever been found upon a spine which could be definitely identified as arising from the peripheral process of a granule cell. In spite of extensive searching, none of the processes to which these spines were attached was large enough to be a peripheral process, or was oriented perpendicular to the mitral cell layer.

The only possible exception to this last statement is shown in Fig. 23. In this single section the degenerating terminal appears to contact 3 separate spines, one of which is connected to a large process containing portions of a Golgi apparatus and of granular endoplasmic reticulum. In serial sections, however, these 3 ‘spines’, together with several others which appeared in adjacent sections, were all found to be parts of
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the same complex spine. This complex spine has a single origin from its parent process, but it then divides into several 'subspines', each of which participates in a separate synapse with the axon terminal, and several of which contain a separate spine apparatus. A semidiagrammatic reconstruction of this unusual spine is shown in Fig. 4. That this spine arises from a granule cell is suggested by its several inclusions, particularly flattened vesicles and multivesicular bodies; as the parent process contains a portion of Golgi apparatus, it may be tentatively identified as the most basal portion of a peripheral process (Price & Powell, 1970b). A possible example of such a complex spine arising from a cell body in Golgi material is shown in Fig. 5 of the account of the morphology of the granule cell (Price & Powell, 1970b). As the portion of the peripheral process which contains the Golgi apparatus could be considered as an extension of the cell soma, however, this example need not invalidate the suggestion made above that the anterior commissure fibres end on somatic spines but not upon spines of the peripheral processes.

Degenerating terminals have also been found on the swollen portions of varicose dendrites (Fig. 22). In single sections these are often difficult to distinguish from spines as they also lack neurotubules and have a flocculent cytoplasm, but in serial sections the varicosities are found never to contain a spine apparatus, and to be directly continuous with smaller shafts that are more typically dendritic.

There is, therefore, considerable evidence that the fibres of the anterior commissure end upon the spines and varicosities of the deep dendrites, and upon the somatic spines of these cells, but not upon the peripheral processes. It is quite possible, however, that these fibres have an additional termination on the short axon cells of the olfactory bulb, especially those which have spiny dendrites, and there is physiological evidence which could be considered to support such a suggestion.

Occasionally degenerating terminals are found in the deeper parts of the external plexiform layer after a lesion of the anterior commissure. Although these are rare,
enough of them have been found to suggest that they are due to the experimental interruption of the anterior commissure. In all instances these degenerating terminals end upon vesicle-containing profiles; these have never been seen to form a reciprocal synapse with the dendrites of the mitral and tufted cells, but the sample was admittedly not large enough to be examined with serial sections. The most probable explanation for these unusually placed terminals of the fibres of the anterior commissure is that they are ending upon the aberrant dendrites which occasionally arise from the superficial side of the perikarya of granule cells, or from the peripheral processes, and which may enter the external plexiform layer; these dendrites closely resemble the deep dendrites in size, and may even appear slightly varicose (Price & Powell, 1970b).

**Fibres from the ipsilateral anterior olfactory nucleus**

The lesions which damaged the anterior olfactory nucleus but which left the olfactory bulb of the same side intact were used to study the termination of the axon collaterals from the anterior olfactory nucleus, which have been shown by Valverde...

![Diagram of the distribution of degenerating terminals](image)

*Fig. 5. Composite map of the distribution of degenerating terminals (x) after a lesion of the anterior olfactory nucleus of the same side of the brain. The degenerating terminals are found in both the granule cell and external plexiform layers, and it should be noted that they are present in the superficial as well as the deep half of the external plexiform layer.*

(1965) to enter the ipsilateral olfactory bulb along with the axons of the anterior commissure from the opposite side. It should be noted that these lesions also interrupt the centrifugal fibres and the fibres of the anterior commissure and that the degeneration found within the olfactory bulb must therefore be attributed to all 3 projections.

In these experiments the optimum survival time was found to be 5 days, as it was for the investigation of the centrifugal fibres. The use of this survival time gave the
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added advantage that the terminal degeneration due to the damage to the anterior commissure had largely disappeared, and that the degenerating terminals in the granule cell layer could be assumed for the most part to be those of the fibres from the ipsilateral anterior olfactory nucleus.

As would be expected from the experiments on the other projections, degenerating axons are found in all layers of the bulb, and degenerating terminals are present in the external plexiform layer and in the granule cell layer. The 'map' of this terminal degeneration in Fig. 5, however, shows that the distribution of this degeneration differs in two ways from what would be expected if it were due merely to the combined damage to the centrifugal fibres and to the anterior commissure: first, there is more degeneration in the granule cell layer than has been found after a lesion of the anterior commissure, and second there are degenerating terminals throughout the external plexiform layer, instead of only in the deep half, as was the case with a pure lesion of the centrifugal fibres in the lateral olfactory tract. This evidence suggests, therefore, that the fibres from the ipsilateral anterior olfactory nucleus terminate in both the granule cell layer and in the external plexiform layer.

In the granule cell layer, degenerating terminals are found forming asymmetrical synapses upon profiles which have flocculent cytoplasm, and lacking neurotubules, but which often contain a spine apparatus, vesicles, mitochondria, and other inclusions (Figs. 24, 30). These fibres from the ipsilateral side therefore appear to end upon spines, and possibly also upon the swollen portions of the varicose dendrites in a manner similar to the terminals of the anterior commissure. Even in single sections, however, an important difference can be seen between these two projections: whereas degenerating terminals of anterior commissure fibres have never been found in synaptic relation to a spine which could be unequivocally identified as arising from a peripheral process, after lesions of the ipsilateral anterior olfactory nucleus, degenerating terminals are frequently found upon such spines (Figs. 28, 29). Although most of these spines, including the ones illustrated, are sessile, this is probably due to the difficulty of sectioning pedunculated spines in continuity with their parent process; in serial sections, degenerating terminals have been found upon pedunculated spines which arise from undoubted peripheral processes (Fig. 6).

After these lesions of the ipsilateral anterior olfactory nucleus, degenerating terminals are also found upon spines of granule cell somata and deep dendrites (Fig. 1), upon the swollen portions of the varicose deep dendrites, and occasionally upon the cell somata themselves (Fig. 27), but these are comparatively rarer than those upon spines of the peripheral processes. While no firm conclusion can be drawn as to the origin of these terminals, it is possible that they are the terminals of the fibres of the anterior commissure, and that the fibres from the ipsilateral anterior olfactory nucleus do not end upon the deeper portions of the granule cell. Alternatively, both groups of fibres might terminate on the deeper portions of these cells.

The postsynaptic profiles in the external plexiform layer which receive degenerating terminals can be definitely identified as gemmules; they contain relatively large, flattened vesicles, arise in a spine-like manner from peripheral processes (Fig. 32), and participate in reciprocal synapses with mitral or tufted cell dendrites (Fig. 33). Many
of these terminals are undoubtedly those of the centrifugal fibres, but the fact that, after these lesions which are closer to the olfactory bulb, degenerating terminals are present in the superficial half of the external plexiform layer, as well as in the deep half, suggests that some of them may also be the terminals of fibres from the anterior olfactory nucleus. With the lesions of the anterior olfactory nucleus, special care had to be taken to ensure that the lesion did not damage the olfactory bulb directly, and thereby interrupt the intrinsic axons of the bulb. Two brains were also studied, however, in which the lesion did encroach slightly upon the olfactory bulb, as well as destroying the anterior olfactory nucleus of that side almost completely; both these animals had survived for 5 days post-operatively. In addition to massive terminal and fibre degeneration in all layers of the olfactory bulbs ipsilateral to these lesions, there are many axon terminals which show no signs of degeneration. While it is possible that a few of these terminals are those of extrinsic fibres which for some reason have not yet degenerated, the great majority of the remaining normal terminals must be intrinsic to the olfactory bulb. The synapses made by these terminals can be divided into two classes which are the same as those found in normal material: those with asymmetrical synaptic membrane thickenings and spheroidal vesicles, and those with
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Symmetrical synaptic thickenings and smaller, flattened vesicles (Price & Powell, 1970c). The terminals associated with asymmetrical synapses are predominantly of the 'pale' type found in normal material and many of them can be shown to be en passant. No quantitative study has been made of these synapses, but a qualitative impression has been obtained that the synaptic vesicles are of the larger spheroidal variety. Because both types of these terminals are found in the external plexiform, granule cell, and periventricular layers, they probably end upon all parts of the granule cells, and possibly also upon the short axon cells.

Discussion

By using the electron microscope to study experimental material, it has been possible to define the precise site and mode of termination of the extrinsic afferent fibres to the olfactory bulb from the cerebral hemisphere (Price, 1969a). Of the techniques available at present, electron-microscopic investigation is the only method by which these results could have been achieved.

All these extrinsic fibres, the centrifugal fibres and the fibres from the anterior olfactory nucleus, on the ipsilateral side, and the fibres which cross in the anterior commissure from the contralateral anterior olfactory nucleus, have been found to terminate on the granule cells of the olfactory bulb (and possibly on other cells in the bulb) by way of asymmetrical synapses; the distribution of these terminals upon the spines, gemmules, dendritic varicosities, and occasionally on the perikarya of the granule cells corresponds precisely with the distribution of synapses with asymmetrical synaptic membrane thickenings and spheroidal vesicles found in normal material (Price & Powell, 1970c). This latter point is considered to be particularly important, as it is often difficult to be sure of the type of synapse formed by a degenerating terminal. It may be mentioned that the other group of afferent fibres to the olfactory bulb, the olfactory nerves from the olfactory mucosa, also form asymmetrical synapses with the dendrites of the mitral and tufted cells, and probably with the periglomerular cells (Andres, 1965; J. L. Price & T. P. S. Powell, unpublished observations). This finding that all the extrinsic fibres form asymmetrical synapses, and that fibres ending in symmetrical synapses must therefore be intrinsic, is analogous to the results which have been obtained in the cerebellum (Eccles, Ito & Szentágothai, 1967; Uchizono, 1967), the cerebral cortex (Jones & Powell, 1970) and in the caudate nucleus (Kemp, 1968).

The process of degeneration and the appearance of degenerating axons and terminals in the olfactory bulb is very similar to that which has been described in other parts of the brain (e.g. Colonnier & Gray, 1962; Colonnier, 1964; Walberg, 1964, 1965; Westrum, 1966; Laatsch & Cowan, 1967). It need not be discussed in detail because there should be little doubt of its validity, and in support of this it may be mentioned that degenerating terminals were never found in normal material, nor on the side contralateral to the lesion, unless the anterior olfactory nucleus or the anterior commissure had been damaged. Furthermore, the distribution of degenerating axons in the olfactory bulb as seen with the electron microscope corresponds precisely to the degeneration which
can be found with the light microscope in material impregnated with the Nauta method.

While the mode of degeneration of the presynaptic profiles is fairly well documented, there is some uncertainty as to the reaction of the postsynaptic profiles contacted by degenerating terminals. Colonnier (1964) has reported that spines can become 'pinched off' and engulfed by glia along with degenerating terminals, but this is questioned by Westrum (1966). In the present material degenerating terminal-spine complexes have been found which in single sections appear to be completely surrounded by glia, but in serial sections the spine has always been found to be still attached to its parent process. Spines or gemmules which receive degenerating terminals often appear to be slightly shrunken and distorted, however. It is possible that the disappearance of spines, in material impregnated by the Golgi method, following deafferentation or sensory deprivation, which has been reported recently (Globus & Scheibel, 1967a, b; Valverde, 1967, 1968; Valverde & Esteban, 1968) could be explained by spine shrinkage or reabsorption rather than by removal of spines by glia.

The optimum survival time for maximal degeneration of axon terminals appears to vary with each fibre pathway, and it is possible that this should be determined in advance for each fibre system which is being investigated experimentally with the electron microscope. It is interesting that of the pathways studied in this investigation the terminals of the thinnest fibres (those of the anterior commissure) degenerated fastest. This is in agreement with other electron-microscopic results (Mugnaini, Walberg & Brodal, 1967) but not with previous light-microscopic observations (van Crevel & Verhaart, 1963a, b). This discrepancy may be more apparent than real as the light-microscopic studies have been principally concerned with the rate of fibre degeneration, whereas electron-microscopic observations relate mainly to the degeneration of axon terminals.

It is important to state that every experimental brain was prepared for light-microscopic examination in order to determine the site and extent of the lesion. None of these lesions interfered with the blood supply to the olfactory bulb, and any brain in which there was direct damage to the olfactory bulb itself was discarded for the purpose of investigation of the extrinsic afferent fibres, although such brains were of use for the indirect investigation of the intrinsic connexions of the bulb. 'Dark dendrites' were found only rarely, were easily recognized, and were present in only a few brains which were less well fixed.

As well as showing that all of the extrinsic afferent fibres end upon the granule cells, the work has made it possible to obtain information on the specific portions of these cells upon which each fibre pathway terminates. It is necessary to point out certain reservations about these results, especially the negative findings. Although almost 50 animals and thousands of ultrathin sections were used in this study, and at least 1000 degenerating terminals were observed, the sample of degenerating terminals which end upon unequivocally identifiable processes, such as a spine undoubtedly arising from a peripheral process, was only 5-10% of this total and must therefore be considered to be relatively small; this is a problem with all electron-microscopic
investigations, because of the small absolute quantity of tissue which can be examined and the difficulty of identification of profiles in ultrathin sections. Serial sections greatly facilitate identification, and often provide more conclusive evidence than could be obtained with single sections, but they also greatly reduce the sample which can be taken. A degenerating terminal of a fibre of the anterior commissure has never been found to end upon a spine of a peripheral process, except for one possible exception in which the spine was very close to the cell soma, but after a lesion of the ipsilateral anterior olfactory nucleus degenerating terminals have been frequently found in synaptic relation to such spines. It has therefore been concluded that the commissural fibres do not end upon the peripheral processes, and that the fibres from the ipsilateral anterior olfactory nucleus do end at this site. Admittedly, however, a larger investigation might show that the difference between the termination of these two groups of fibres is not as sharp as has been stated. Similarly, degenerating terminals of the centrifugal fibres have been found to end only upon the gemmules of the peripheral processes in the deep half of the external plexiform layer, but after the larger lesions which damage the anterior olfactory nucleus degeneration of terminals is found throughout the whole depth of the external plexiform layer, suggesting that the fibres from this nucleus end upon the gemmules, as well as on the spines on more proximal parts of the peripheral processes. It is possible, however, that the additional terminal degeneration found after a lesion of the anterior olfactory nucleus is due to centrifugal fibres which degenerate after a lesion close to the olfactory bulb, but not after one farther away. Experimental investigations have shown that after a lesion of the lateral olfactory tract there is a large number of degenerating axons in the glomerular layer, but no degenerating terminals have been found in the glomeruli or amongst the periglomerular cells. As the degenerating fibres cannot be merely fibres of passage at this level in the bulb, the problem of the termination of these axons remains unsolved.

The emphasis which has been placed upon the granule cells is justified by the finding that all the extrinsic afferent fibres to the olfactory bulb, except for the olfactory nerves, terminate on various parts of these cells, and that, outside the glomerular formations, the granule cells appear to be the only cells which act directly upon the mitral and tufted cells. The only type of synapse which is found on the mitral and tufted cells in the external plexiform and mitral cell layers is the reciprocal synapse. The extrinsic fibres may also terminate upon the other types of interneurons in the olfactory bulb: the short axon cells, and the periglomerular (or external granule) cells.

The extensive ramification of the centrifugal axons among the periglomerular cells suggests that these fibres may terminate on these cells, although no experimental support for this has been obtained. In regard to the short axon cells, the findings of Yamamoto, Yamamoto & Iwama (1963) and of Callens (1965) that a synchronous volley in the anterior commissure can either inhibit the mitral cells, or release them from inhibition caused by a volley in the lateral olfactory tract, were taken by these authors to suggest that the fibres of the anterior commissure contact two different types of interneurons in the deep layers of the olfactory bulb. The first of these might be the granule cells, which may inhibit the mitral cells, and the second could be the short axon cells, in turn inhibiting the granule cells. Although no degenerating
terminals of the anterior commissure fibres have been found ending directly upon the somata of the short axon cells, or on other processes which could be identified as arising from the short axon cells, such a termination cannot be excluded by the present evidence.

These experimental investigations have also provided indirect evidence on the intrinsic connexions of the bulb. Thus, all the axon terminals with symmetrical synaptic membrane thickenings and flattened vesicles, and a large number of endings with asymmetrical thickenings and spheroidal vesicles have been found to remain intact even after complete interruption of the extrinsic connexions from the cerebral hemisphere. These connexions have already been discussed (Price & Powell, 1970c) and it need only be mentioned here that both types of intrinsic fibres are found on all levels of the granule cells: peripheral processes, cell somata, and deep dendrites. Those fibres which form asymmetrical synapses, and are presumed to be the axon collaterals of the mitral and tufted cells, contact the gemmules, spines, and dendritic varicosities, while the symmetrical synapses, presumed to be formed by the axons of short axon cells, are found on the shafts of the peripheral processes and deep dendrites, and on the cell somata.

In spite of the above reservations, there is considerable evidence that the different groups of afferent fibres to the olfactory bulb have a differential termination on the different parts of the granule cells, the fibres of the anterior commissure ending upon the spines of the deep dendrites and the cell somata, those from the ipsilateral anterior olfactory nucleus upon the spines of the peripheral processes, and possibly on the gemmules, and the centrifugal fibres on the gemmules (Fig. 7). There are several other examples of differential patterns of termination of afferent fibres on specific cells in the brain, and it may be suggested that this sort of differential termination is present throughout the central nervous system, and that on certain cells in 'cortical' structures these terminations are arranged in laminae. One of the earliest suggestions of a pattern of this type was made by Lorente de Nó (1934) and experimental evidence for such a termination of afferent fibres on pyramidal cells of the hippocampus has been provided (Blackstad, 1956; Raisman, Cowan & Powell, 1965). There is also a similar arrangement in the pyriform cortex and the anterior olfactory nucleus where the fibres of the lateral olfactory tract end in the superficial half of the plexiform layer, and the association fibres end in the deep half, so that the different fibre pathways contact different parts of the apical dendrites of the pyramidal cells of that cortex (White, 1965; Heimer, 1968; Price & Powell, 1970a). In the more complex neocortex the organization is not so clear, but there is evidence that cortical afferent fibres may end upon specific parts of the apical dendrites of the pyramidal cells in this site as well (Globus & Schciebel, 1967a, b; Valverde, 1967; Valverde & Estéban, 1968; Jones & Powell, 1970). The fibres ending upon the Purkinje cells of the cerebellar cortex also have differential terminations on the various parts of these cells, although there is no laminar pattern of termination (Eccles et al. 1967; Fox, Hillman, Siegesmund & Dutta, 1967). The physiological interpretation of these patterns of termination must at present remain speculative. Calculations based on a mathematical neuron model, however, have shown that spatially different synaptic inputs to the dendritic tree of a neuron would
be expected to have significantly different effects on the membrane potential of the cell soma (Rall, 1967), and Diamond & Yasargil (1969) have suggested that 'the integrative activity of the cell should not be viewed simply as a single mass affair' but 'in at least two parts, dendritic integration, and the final somatic/IS integration'.

In relation to the granule cells of the olfactory bulb it would be of interest to compare the precise effects upon the reciprocal synapses of stimulation of the anterior commissure and of the centrifugal fibres. If the site of termination of these fibres on the granule cells which has been presented here is correct, any influence upon the reciprocal synapses from the anterior commissure would have to be conducted through
the peripheral processes; especially if this conduction is passive, these influences might be expected to be modulated by synaptic influences from the fibres from the ipsilateral anterior olfactory nucleus, the axon collaterals of the mitral and tufted cells, and the intrinsic axons which form the symmetrical synapses. The centrifugal fibres, on the other hand, would act directly upon the gemmules, and thereby on the reciprocal synapses; their action might be either to activate the granule to mitral cell half of these synapses, or, in a manner similar to that proposed for presynaptic inhibition (Eccles, 1964), to reduce the response of this half of the reciprocal synapses to influences from other sources.

In conclusion, this investigation again shows it is essential to correlate the results of electron-microscopic investigations on experimental material with those obtained from a similar examination of normal material, and with those from light-microscopic studies of experimental material prepared with the Nauta method, and of normal material prepared with the Nissl and Golgi methods. Only in this way can a complete understanding of the structure and connexions of a specific cell, or of a specific part of the brain, be obtained. Although we are still far from understanding the structure or the function of the olfactory bulb, it can be predicted that the granule cells play a predominant role in the integrative processes which occur within the bulb. They are the final common path through which act not only the extrinsic influences from the more caudal parts of the brain, but also the recurrent influences between different mitral and tufted cells, and the modulating influences from the short axon cells. Conclusive answers to the questions raised here can be provided only by further physiological investigations, but it would also be important to have quantitative information on the various intrinsic and extrinsic connexions. As all these systems which end in the external plexiform and granule cell layers must act through the granule cells, the final action on the mitral and tufted cells will depend not only upon which systems are activated, but also on the relative strength of the synaptic action produced by each of them. As yet, no information of this sort is available.

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Afferent fibres to the olfactory bulb


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**ABBREVIATIONS ON PLATES**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Legend</th>
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<tr>
<td>ax</td>
<td>axon</td>
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<tr>
<td>g</td>
<td>gemmule of granule cell</td>
</tr>
<tr>
<td>gr</td>
<td>granule cell</td>
</tr>
<tr>
<td>m</td>
<td>mitral cell, soma or dendrite</td>
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<td>p</td>
<td>peripheral process of granule cell</td>
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<td>r</td>
<td>reciprocal synapse</td>
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<td>sp</td>
<td>spine</td>
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<td>v</td>
<td>dendritic varicosity</td>
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Fig. 8. Axon terminal with asymmetrical synaptic thickening in the external plexiform layer of the olfactory bulb showing early degeneration, 3 days after a lesion of the ipsilateral anterior olfactory nucleus. × 39000.

Fig. 9. Axon terminal of centrifugal fibre forming an asymmetrical synaptic contact with 2 gemmules and which shows typical degeneration 5 days after a lesion confined to the lateral olfactory tract. × 43000.

Fig. 10. Two degenerating terminals of centrifugal fibres showing considerable shrinkage and distortion. The long sinuous "tail" (arrow) continuous with the degenerating terminal on the left may be the adjoining preterminal axon; the curved form of this tail is very characteristic of this stage of degeneration. × 30000.

Fig. 11. Late stage of degeneration of terminal following a lesion of the anterior commissure. × 40000.

Fig. 12. Late stage of degeneration of axon terminal with marked glial reaction. The remnants of the degenerating terminal may be seen making synaptic contacts with two spines (above), but a third spine (below) shows an exposed postsynaptic membrane thickening (arrow) which is directly apposed to the membrane of the glial process. × 34000.

Figs. 13, 14. Two degenerating terminals which have apparently retracted away from a portion of postsynaptic membrane thickening to which they were related (arrows), in the external plexiform layer following a lesion of the lateral olfactory tract. Both × 35000.
Afferent fibres to the olfactory bulb
Fig. 15. Degenerating myelinated centrifugal axon in the granule cell layer of the olfactory bulb. × 7200.

Fig. 16. Degenerating centrifugal fibre close to its termination in the external plexiform layer. In serial sections the 3 swellings on this axon were seen to make unequivocal synapses en passant. × 15000.

Fig. 17. Preterminal portion of degenerating axon making 3 synapses (arrows), in the external plexiform layer following a lesion of the ipsilateral anterior olfactory nucleus. × 27000.

Fig. 18. Degenerating myelinated axon and terminal in the granule cell layer. In serial sections continuity was seen between the axon and the terminal, and the myelin sheath around the axon became progressively thinner, as if the fibre was approaching a node of Ranvier. × 22 500
Fig. 19 A–D. Electron micrographs of a sequence of serial sections to show the identification of the process as a gemmule with which a degenerating centrifugal axon terminal is making synaptic contact. Note that this gemmule can be traced in continuity, through a peripheral process, with a second gemmule which participates in a reciprocal synapse with a dendrite (below). A drawing of a reconstruction of this series is shown in Fig 2. × 18000.
Afferent fibres to the olfactory bulb
Fig. 20. Two degenerating terminals in close proximity to each other after a lesion of the anterior commissure. In serial sections 3 other terminals were found in the immediate vicinity. \(\times 23000\).

Fig. 21. Degenerating axon terminal making synaptic contact with a spine arising from the perikaryon of a granule cell. Three days after a lesion of the anterior commissure \(\times 32000\).

Fig. 22. A, Degenerating terminal of axon of the anterior commissure forming a synapse with deep dendrite of a granule cell; the varicose nature of this dendrite is seen in the electron micrograph (B) taken of another section of the series through this dendrite \(\times 26000\).

Fig. 23. A degenerating terminal of a fibre of the anterior commissure making synaptic contact with what appeared in this section to be 3 separate spines, one of which is arising from a process near to its cell body. Serial sections, however, showed that these spines all arose from the single pedicle. A semidiagrammatic representation of this spine is given in Fig. 4. \(\times 25000\).
Afferent fibres to the olfactory bulb
Fig. 24. Degenerating terminal, 5 days after a lesion of the ipsilateral anterior olfactory nucleus, forming an asymmetrical synapse with a spine filled with flattened vesicles in the granule cell layer. $\times 29000$.

Fig. 25 A, B. Electron micrographs of 2 sections of a series to show degenerating terminal of a fibre of the anterior commissure making synaptic contact with a spine arising from a dendrite of medium size in the granule cell layer. The medium size of this dendrite is clearly shown in B. $\times 24000$.

Fig. 26 A, B, C. Electron micrographs of 3 sections of a series to show that the spine with which the degenerating terminal is making synaptic contact arises from a varicose dendrite. The varicose nature of this dendrite was shown even more clearly in the adjoining sections, in which the dendrite could be seen to taper in both directions to a diameter of approximately 0.3 $\mu$m. $\times 35000$.

Fig. 27. A degenerating terminal, following a lesion of the anterior olfactory nucleus of the same side of the brain, ending in an asymmetrical synapse upon the soma of a granule cell. $\times 35000$. 
Afferent fibres to the olfactory bulb
Figs. 28, 29. Degenerating terminals following lesions of the ipsilateral anterior olfactory nucleus ending upon spines arising from peripheral processes of granule cells. Fig. 28, \( \times 22,000 \); Fig. 29, \( \times 25,000 \).

Fig. 30. Degenerating terminal due to damage of the anterior olfactory nucleus ending upon a spine filled with flattened vesicles. \( \times 27,000 \).

Fig. 31. Degenerating terminal resulting from a lesion of the anterior olfactory nucleus ending upon a spine arising either from the cell soma, or from the proximal part of a deep dendrite of a granule cell. \( \times 25,000 \).

Figs. 32, 33. Degenerating terminals after the same type of lesion ending upon gemmules in the external plexiform layer. In Fig. 32 the gemmule can be seen arising from a peripheral process, and in Fig. 33 the gemmule is participating in a reciprocal synapse with a mitral cell dendrite. Fig. 32, \( \times 23,000 \); Fig. 33, \( \times 26,000 \).