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The afferent connexions of the nucleus of the horizontal limb of the diagonal band

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INTRODUCTION

In the preceding paper (Price & Powell, 1970*a*) experimental evidence was presented to show that the centrifugal fibres of the olfactory bulb arise in the nucleus of the horizontal limb of the diagonal band, and in this paper the results of an investigation into the afferent connexions of this nucleus will be presented. From what is known of the organization of the central and centrifugal pathways in other sensory systems, it would be reasonable to predict that this part of the nucleus of the diagonal band receives fibres from one or more of the structures in which synaptic relays occur on the olfactory pathway. Such structures include, first, the olfactory bulb, secondly, those sites to which the bulb projects and finally, those areas which in turn receive third-order olfactory connexions (Powell, Cowan & Raisman, 1963, 1965; Cowan, Raisman & Powell, 1965). In the present study, therefore, lesions have been placed in most parts of the olfactory pathways and the resultant distribution of fibre and terminal degeneration examined in relation to the nucleus of the horizontal limb of the diagonal band (Price, 1969).

MATERIAL AND METHODS

The brains of 75 rats were available for this study; many of them were also used for the study of the origin of the centrifugal fibres (Price & Powell, 1970*a*) and the details of the operative procedure, fixation, and histological techniques have been given in the preceding paper. It is sufficient to state here that the distribution of the axonal and terminal degeneration, 4–7 d after operation, has been studied in frozen sections impregnated by the methods of Nauta & Gygax (1954) and of Fink & Heimer (1967).

RESULTS

Lesions of the olfactory bulb and anterior olfactory nucleus

Two examples will be described from this group of experiments, but only the possible projection to the nucleus of the horizontal limb of the diagonal band will be considered. In the first of these experiments (R 761) there is a small lesion restricted to the dorsal surface of the olfactory bulb which does not encroach upon any part of the anterior olfactory nucleus, while in the second (R 764) there is a much larger lesion which destroys the entire olfactory bulb and most of the olfactory peduncle, including all but the most caudal regions of the anterior olfactory nucleus.

In both of these brains, which are cut sagittally, there is well-stained terminal degeneration immediately superficial to the nucleus of the diagonal band, rostrally in the olfactory tubercle, and caudally in the nucleus of the lateral olfactory tract and the cortical and medial amygdaloid nuclei. In R764 there is also fibre degeneration in the medial forebrain bundle lateral and anterior to the nucleus, as well as in the thalamus and hypothalamus, but in neither brain is there any degeneration within the nucleus itself. In agreement with previous reports, therefore, there is no direct connexion with the 'nucleus of the horizontal limb of the diagonal band' from the olfactory bulb or the anterior olfactory nucleus.

ABBREVIATIONS

AC	Anterior commissure	LH	Lateral habenular nucleus
AOB	Accessory olfactory bulb	LOT	Lateral olfactory tract
AON	Anterior olfactory nucleus	M	Medial nucleus of amygdala
B	Basal nucleus of the amygdala	MC	Mitral cell
C	Central nucleus of the amygdala	MD	Medio-dorsal nucleus of thalamus
Co	Cortical nucleus of the amygdala	MFB	Medial forebrain bundle
EPL	External plexiform layer of olfactory bulb	NOT	Nucleus of the lateral olfactory tract
GCL	Granule cell layer of olfactory bulb	OT	Olfactory tubercle
GL	Glomerular layer of olfactory bulb	PC	Pyriform cortex
GC	Granule cell	S	Corpus striatum
HDB or Nucleus HLDB	Nucleus of the horizontal limb of the diagonal band	SM	Stria medullaris
		VDB	Nucleus of the vertical limb of the diagonal band

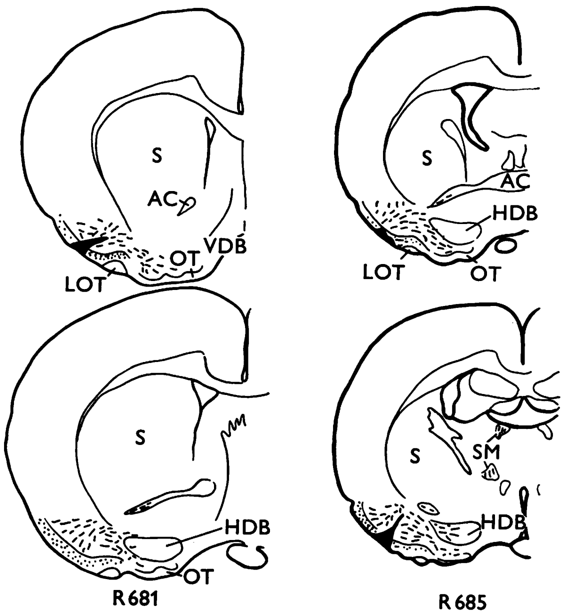
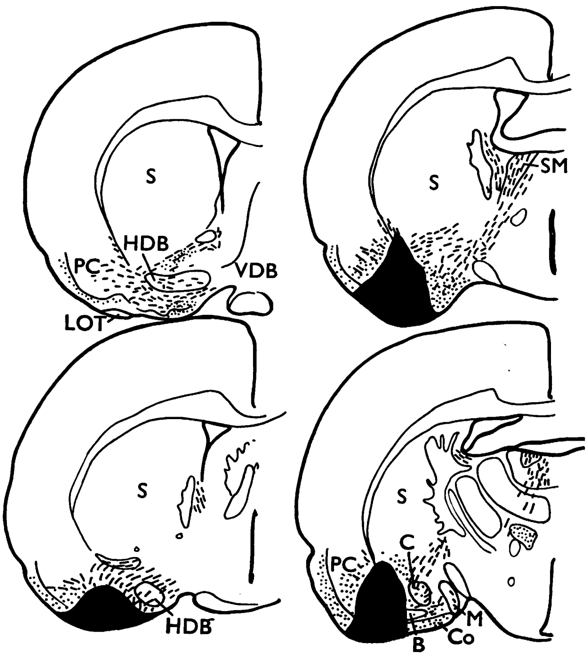


Fig. 1. The site and extent of the lesions in the pyriform cortex in R681 and R685. In this and subsequent figures the lesion is shown in solid black, the fibre degeneration in dashes and the terminal degeneration by stipple. It may be noted that although degenerating fibres pass through the lateral part of the nucleus of the horizontal limb of the diagonal band, there is no terminal degeneration in this nucleus.

Lesions of the pyriform cortex, amygdala and olfactory tubercle

Five experiments will be considered as representative of a group of brains with lesions of these primary olfactory projection areas. Two of these (R681) and (R685) have lesions restricted to the pyriform cortex; while in the third (R730) a stereotaxically placed lesion has damaged the amygdala as well as a portion of the overlying pyriform cortex; two further brains (R661 and R679) contain superficial lesions of the olfactory tubercle.



R730

Fig. 2. The lesion in the amygdala of experiment R730, together with the resulting fibre and terminal degeneration. Degenerating fibres are present in the nucleus of the horizontal limb of the diagonal band, but there is no unequivocal terminal degeneration.

In R681 the lesion is small and lies opposite the middle of the lateral olfactory tract, rostral to the level of the anterior commissure and the optic chiasma; it does not encroach upon the lateral olfactory tract nor on the neocortex (Fig. 1). The lesion in R685 is similarly restricted to the pyriform cortex, but it is larger and more caudal, lying posterior to the level of the anterior commissure at the caudal end of the olfactory tract (Fig. 1). In both of these brains degenerating fibres pass medially from the lesion into the lateral fascicle of the medial forebrain bundle and then continue rostrally to the anterior olfactory nucleus or caudally to the thalamus and hypothalamus. These fibres are restricted to the medial forebrain bundle, however, and there is no terminal or preterminal degeneration within the nucleus of the horizontal limb of the diagonal band. The only degenerating fibres in the nucleus are in that part which coincides with the medial forebrain bundle; large areas of the nucleus medial and anterior to the bundle are clear of degeneration.

A very ~~similar~~ pattern of degeneration is found after the larger lesion of the amygdala and posterior pyriform cortex in R730 (Fig. 2). All parts of the amygdala which project into the ventral amygdalofugal pathway, i.e. the baso-lateral nuclei (Cowan *et al.* 1965), have been destroyed in this brain, as well as most of the cortical nucleus. A large number of degenerating fibres pass rostrally from the lesion into the medial forebrain bundle, and in addition to those seen after lesions restricted to the pyriform cortex, degenerating fibres also pass to the nucleus accumbens, to the postero-medial olfactory tubercle, to the bed nucleus of the stria terminalis, and to the most anterior part of the vertical limb of the diagonal band. The latter fibres do not enter the septum, but instead pass to the anterior hippocampal rudiment where

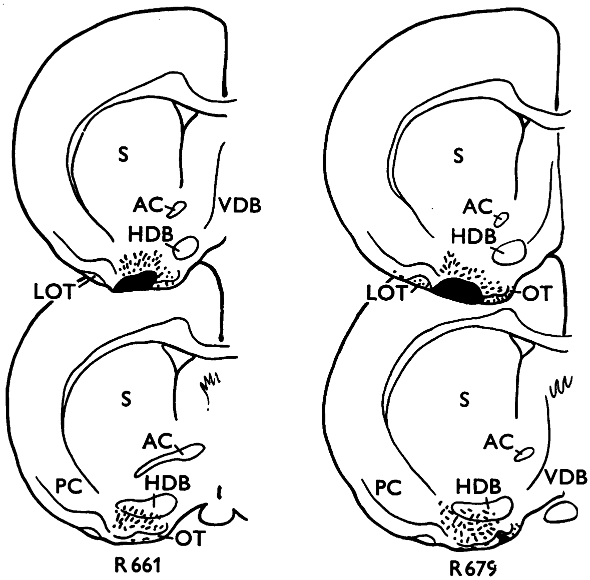


Fig. 3. The extent of the damage to the olfactory tubercle and the resulting fibre degeneration in experiments R661 and R679.

they terminate. Terminal degeneration is particularly well stained in the nucleus accumbens, dorsal to the nucleus of the horizontal limb of the diagonal band, and in the postero-medial olfactory tubercle immediately ventral to the nucleus, but there is no terminal or preterminal degeneration within the nucleus. Because of the large number of fibres passing through this region, degenerating fibres are present in most parts of the nucleus, but they appear to be passing across it, and there is no 'pericellular' fibre degeneration, or any other indication of terminal degeneration. It would thus appear that the degeneration which is present is entirely due to fibres of passage.

In experiments R661 and R679 (Fig. 3) in which there are lesions of the olfactory tubercle there is again degeneration in the medial forebrain bundle, but this is not related to the nucleus of the horizontal limb of the diagonal band. The lesion in

R661 is confined to the lateral and central parts of the tubercle, with a slight encroachment into the ventral part of the medial forebrain bundle, while in R679 the superficial damage is larger and extends caudally to the extreme postero-medial corner of the tubercle and laterally to involve the medial edge of the lateral olfactory tract; in the latter experiment there is also a large area of necrosis in the corpus striatum due to the damage of blood vessels passing through the olfactory tubercle, but this does not affect the present investigation. In both brains degenerating fibres can be seen passing dorsally from the tubercle in tight fascicles to enter all parts of the medial forebrain bundle, and in those areas where the medial forebrain bundle and the diagonal band nucleus overlap, therefore, there are fragmented fibres within the nucleus. These fibres are not considered to terminate here, however, because they are not found in all parts of the nucleus, and because there is no indication of terminal or pre-terminal degeneration.

From these experiments it appears unlikely that the pyriform cortex, the amygdala, or the olfactory tubercle project to the nucleus of the horizontal limb of the diagonal band. However, because many of the axons from each of these areas enter the medial forebrain bundle, and because of the close relationship of this fibre pathway to the nucleus, such a pathway cannot be completely excluded.

Lesions of the hypothalamus

Lesions have been placed stereotaxically in various parts of the hypothalamus in six brains, and three of these (R755, R756 and R765) will be described in detail. In R765 the total rostral projection from the hypothalamus degenerated, for in this brain the medial forebrain bundle has been disrupted just behind the junction between the pre-optic area and the anterior hypothalamus (Fig. 4). The electrode track passes through the fornix and the anterior thalamic nuclei, well lateral to the stria medullaris, and the lesion destroys most of the area between the ventral tip of the internal capsule and the optic tract. The initial course of the stria medullaris is damaged in its caudal half, although the rostral half is intact. Degenerating fibres therefore fill the ipsilateral stria medullaris and lateral habenular nucleus, but it is of interest that no fibres cross to the opposite side through the habenular commissure. Rostral to the lesion on the experimental side, the medial forebrain bundle is filled with degenerating fibres; most of these pass along the medial side of the nucleus of the horizontal limb of the diagonal band into the vertical limb of the diagonal band and thence to the septum. Lateral to this fibre bundle, however, other degenerating fibres continue in the medial forebrain bundle to the horizontal part of the diagonal band. Here they extend beyond the limits of the medial forebrain bundle and fill the nucleus completely (Fig. 8); whereas in the vertical limb of the diagonal band the fibres are disposed in parallel rows, in the nucleus of the horizontal limb there is a definite pericellular arrangement of the fibres, as well as terminal degeneration. A smaller number of fibres pass rostral to the nucleus, and thence can be followed in the medial forebrain bundle as far as the anterior limit of the olfactory tubercle.

The lesions in R755 and R756 are caudal and medial to that in R765 (Fig. 4). The electrode track in R756 again passes through the anterior thalamic nucleus, and in this experiment is immediately lateral to the stria medullaris, but the stria itself is unharmed. The medial forebrain bundle is damaged throughout the extent of the lesion, but it is destroyed completely only at about the mid-point of the rostro-caudal extent of the hypothalamus. Most of the damage is limited to the lateral hypothalamic area although the lateral margin of the ventro-medial hypothalamic nucleus may be involved. The lesion in R755 is very similar to this, except that it is smaller and still

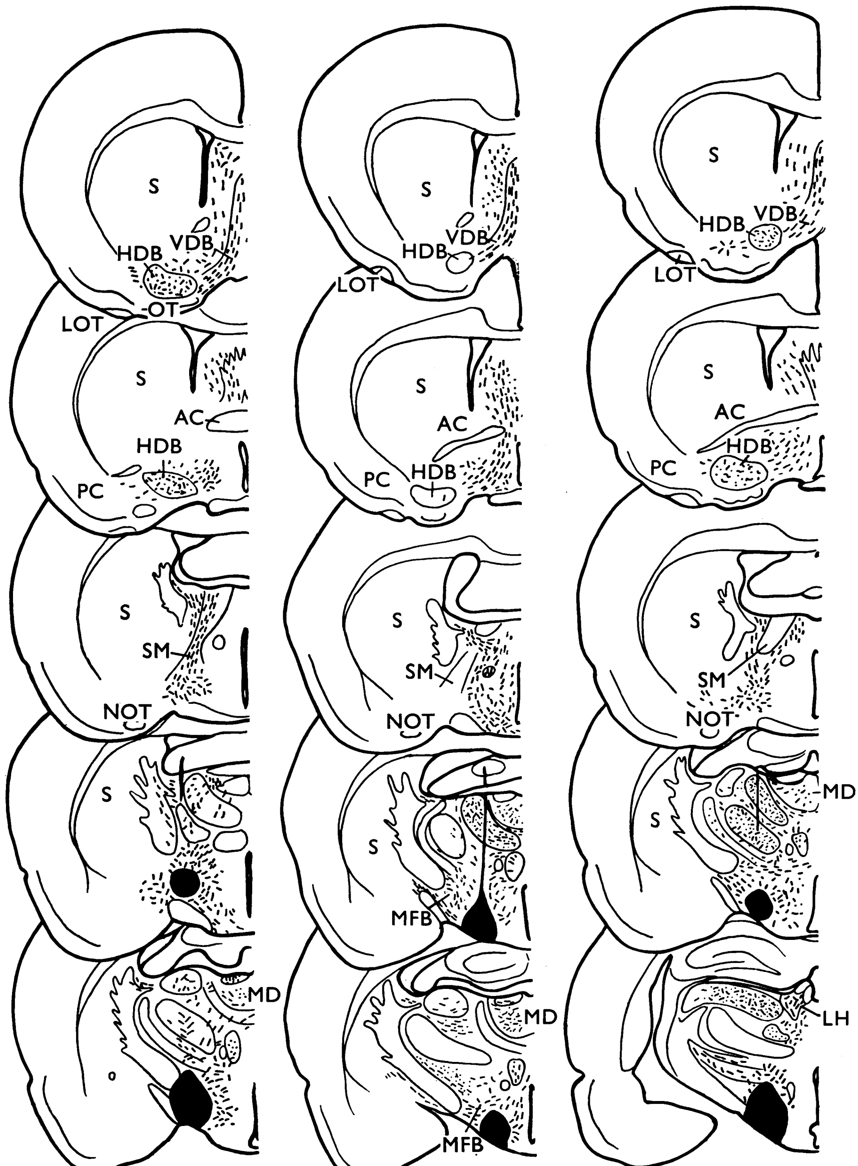


Fig. 4. The positions of the stereotaxically placed lesions and the resulting degeneration in R765, R755 and R756. It may be noted that only when the medial forebrain bundle was damaged in the first and last of these experiments is there terminal degeneration in the nucleus of the horizontal limb of the diagonal band.

further medial, so that the medial forebrain bundle is not involved in the area of damage. A larger part of the ventro-medial nucleus is damaged, but only a relatively small portion of the lateral hypothalamic area. Both of these brains contain well-stained degeneration throughout the thalamus and hypothalamus; the stria medullaris is almost clear, except for a few degenerating fibres in R 755, but the lateral habenular nucleus is filled with degeneration which appears to be due to the thalamic damage. There are also many degenerating fibres in the vertical limb of the diagonal band and the septum, but only in R 756, in which the medial forebrain bundle was damaged, has a significant amount of degeneration occurred in the more lateral parts of the medial forebrain bundle or the nucleus of the horizontal limb of the diagonal band. The latter degeneration closely resembles that seen in R 765, and again a few fibres continue through the nucleus in the medial forebrain bundle as far as the rostral tip of the olfactory tubercle. In R 755, with no direct damage to the medial forebrain bundle, there is very little, if any, degeneration within the nucleus, or in front of it, although there is intense degeneration elsewhere in the same sections.

It can be concluded, therefore, that there are afferent fibres to the nucleus of origin of the centrifugal fibres to the olfactory bulb which pass rostrally in the medial forebrain bundle from the hypothalamus or regions caudal to it. The exact site of origin of these fibres has not yet been determined, but the fact that a lesion of the medial hypothalamus which does not injure the medial forebrain bundle (R 755) does not result in degeneration within the nucleus suggests that they do not come from the medial hypothalamic nuclei. Another brain (R 735) in which a small lesion has disrupted the medial forebrain bundle at the level of the fasciculus retroflexus also contains degeneration within the nucleus, which further suggests that some of these fibres may arise as far caudal as the midbrain.

Lesions of the nucleus of the horizontal limb of the diagonal band

Of the 15 experiments in which lesions have been placed in this nucleus itself, two will be considered in detail (R 776, R 733), as well as one brain with a lesion immediately anterior to the nucleus (R 715), as they provide evidence of commissural connexions between the nuclei of the two sides. In R 776 a large lesion was placed stereotaxically in the ventral striatum and dorsal substantia innominata, extending into the dorsal part of the medial forebrain bundle (Fig. 5). The nucleus of the horizontal limb of the diagonal band is almost completely destroyed, except for a few cells on its ventral aspect. The posterior limb of the anterior commissure is disrupted, although the anterior limb is unaffected; the olfactory tubercle is undamaged. A fuller description of this lesion, with illustrations, was given in the previous paper (Price & Powell, 1970*a*). The lesion in R 733 is smaller, but more ventral, and as well as destroying a large part of the nucleus of the horizontal limb of the diagonal band, has damaged the postero-medial part of the olfactory tubercle; the anterior commissure is only very slightly involved (Fig. 5). The degeneration in these brains need not be described in detail but it may be noted that degenerating fibres enter the stria medullaris and pass caudally in its lateral part (Fig. 6). The majority of these fibres terminate in the lateral habenular nucleus of the same side, but many of them cross in the habenular commissure to reach the lateral habenular nucleus of the opposite side, or continuing rostrally through the stria medullaris (Fig. 7), to terminate in the contralateral nucleus of the horizontal limb of the diagonal band. This nucleus is filled completely with degenerating fragments which have a definite pericellular appearance and which are coarser than the degenerating afferent fibres from the ipsilateral hypothalamus (Fig. 9). As with the

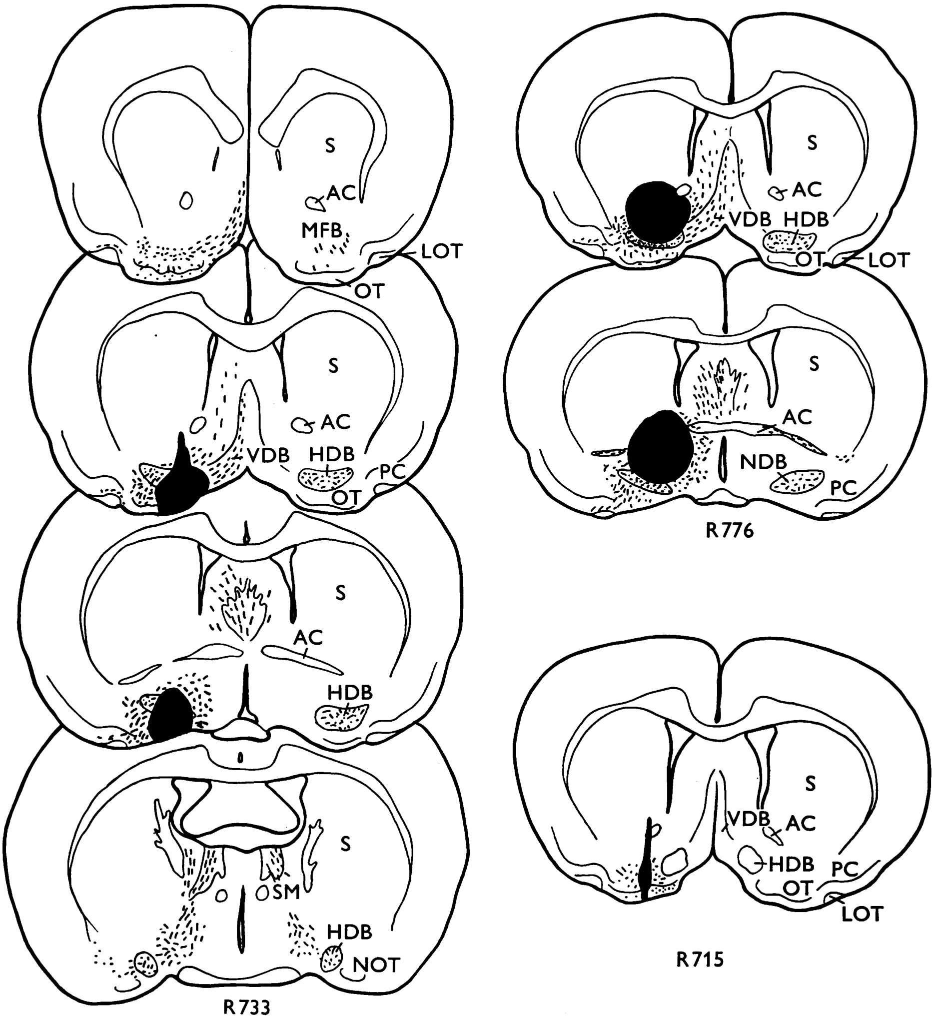
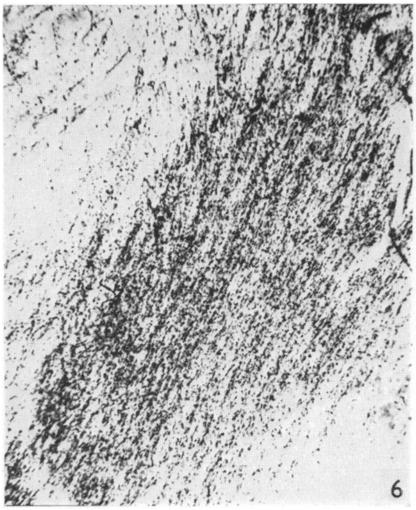
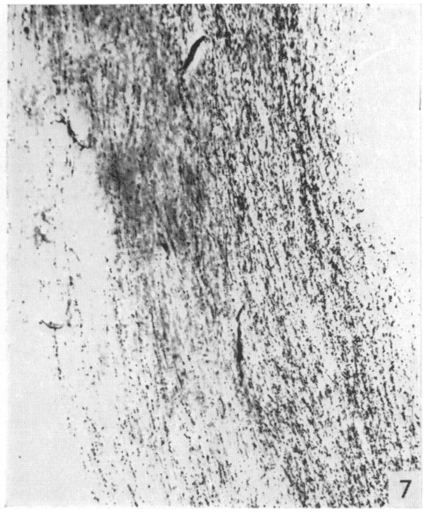
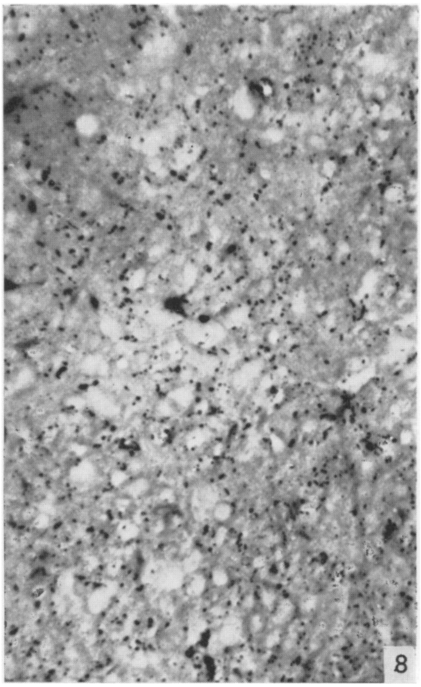


Fig. 5. Experiments R 733 and R 776 show that damage of the nucleus of the horizontal limb of the diagonal band of one side results in terminal degeneration within the same nucleus of the opposite side. In experiment R 715 the damage to the medial forebrain bundle and olfactory tubercle antero-lateral to the nucleus of the horizontal limb of the diagonal band does not cause degeneration of commissural fibres to the opposite nucleus.

ipsilateral projection from the hypothalamus, a few fibres continue rostrally in the medial forebrain bundle as far as the anterior limit of the olfactory tubercle. The commissural fibres have been found to degenerate in every experiment in which the nucleus of the horizontal limb of the diagonal band has been damaged, but not after







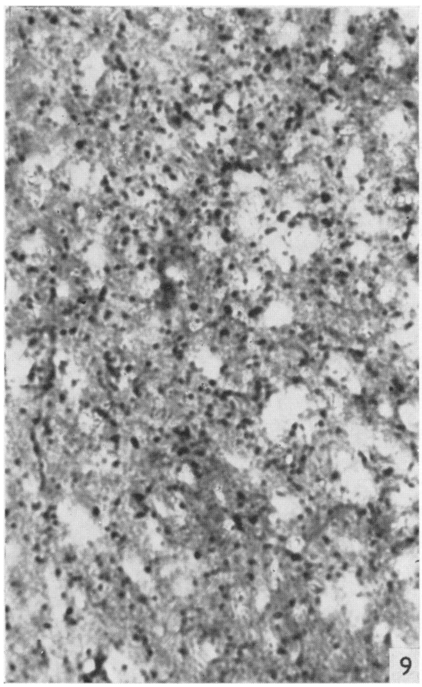


Fig. 6, 7. Photomicrograph to show degeneration of fibres ascending in the stria medullaris on the side of a lesion of the nucleus of the horizontal limb of the diagonal band (Fig. 6), and the less intense degeneration of commissural fibres descending in the stria medullaris of the contralateral side (Fig. 7). ×95.

Fig. 8. Photomicrograph of terminal degeneration within the nucleus of the horizontal limb of the diagonal band after a lesion of the hypothalamus and medial forebrain bundle on the ipsilateral side. ×230.

Fig. 9. The terminal degeneration of commissural fibres in the nucleus of the horizontal limb of the diagonal band after a lesion placed in the same nucleus on the opposite side. ×230. All stained by the Fink–Heimer method.

lesions of the medial forebrain bundle either anterior or posterior to the nucleus (except, in the latter case, when the stria medullaris has been directly involved). A lesion anterior to the nucleus is seen in R715 (Fig. 5); in this experiment the anterior limb of the anterior commissure and the medial forebrain bundle were severely damaged immediately rostral and lateral to the nucleus. Several degenerating fibres are present in the ipsilateral stria medullaris, but they all terminate in the lateral habenular nucleus, and none of them crosses to the opposite side. Of the experiments with lesions posterior to the nucleus, R765 is particularly interesting because this lesion appears to have cut those fibres which project to the ipsilateral habenula, but not the commissural fibres. This indicates that there are two quite separate sets of fibres, and that the commissural component of the stria medullaris enters the stria a little rostral to those fibres which remain on the same side.

DISCUSSION

The results of the experiments which have been described indicate that the nucleus of origin of the centrifugal fibres to the olfactory bulb, the nucleus of the horizontal limb of the diagonal band, receives ascending connexions from the hypothalamus and midbrain of the same side, and commissural connexions from the same nucleus of the opposite side: there do not appear to be any afferent fibres to this nucleus from other parts of the olfactory system. As the validity of these statements depends to a certain extent upon the criteria adopted for the interpretation of *terminal* degeneration, it is necessary to consider these technical points before discussing the significance of the findings. A recurring problem in experimental neurohistological research with the light microscope has been the differentiation of terminal degeneration within an area or nucleus from fragmenting fibres which are passing through that region. This problem is particularly difficult in the basal forebrain areas because the medial forebrain bundle and other diffuse fibre pathways pervade those parts of the brain; as the nucleus of the horizontal limb of the diagonal band lies within and immediately adjacent to the medial forebrain bundle, an unequivocal determination of its afferent connexions is probably impossible with the light microscope.

In the interpretation of the nature of the degeneration in the nucleus of the diagonal band, certain features of *both* the distribution and the appearance of the fragments of degenerating axons and terminals have been taken into account. In regard to the distribution it has been considered important whether the degeneration extends throughout the nucleus, especially into those portions which do not coincide with the medial forebrain bundle, or whether it is only within the limits of the bundle. In the present experiments, degenerating fibres have been found in the medial forebrain bundle after lesions of the pyriform cortex or olfactory tubercle, but these fibres are always restricted to the bundle itself, and leave large areas of the nucleus medial to the bundle free of any degeneration. Although this could represent a projection to the lateral parts of the nucleus only, the same distribution of degeneration is seen after several lesions in different parts of the pyriform cortex and olfactory tubercle, and there is never any indication of terminal or preterminal degeneration. It seems more reasonable to interpret this degeneration as due to the fibres which are known to project from the pyriform cortex and olfactory tubercle to the thalamus and hypothalamus in the medial forebrain bundle (Powell *et al.* 1963, 1965). It may also be noted that there is no noticeable decline in the number of degenerating fibres as they pass through the region occupied by the nucleus of the horizontal limb of the diagonal band.

The Nauta–Gygax method (1954), and especially its recent modification by Fink & Heimer (1967) will stain degenerating terminals directly; in our experience the Fink–Heimer method does not give a qualitatively different result from the Nauta–Gygax method, and any terminal degeneration stained by it can also be found in well-stained Nauta–Gygax material. For example, fine degenerating fibres in layer I of the neocortex after lesions of the cortex of the opposite side have been found with the Nauta–Gygax method (Jones & Powell, 1968) although this is usually given as an example of the increased sensitivity of the Fink–Heimer method (Heimer, Ebner & Nauta, 1967). The Fink–Heimer method, however, does stain degenerating terminals much more intensely and also produces a lighter background against which degeneration shows up more clearly, so that it is possible to be more confident about the result. Four to seven days after lesions which damage the amygdala, there is intense terminal degeneration in the postero-medial part of the olfactory tubercle, and in the nucleus accumbens, but none in the adjoining nucleus of the horizontal limb of the diagonal band. This absence of terminal degeneration, taken together with the fact that the fibres are not arranged in a 'pericellular' manner, but are approximately parallel to each other, suggests that the degeneration found within the nucleus is due to fibres passing to adjoining structures. Because fragmenting fibres are seen in almost all parts of the nucleus of the horizontal limb of the diagonal band, however, a projection from the amygdala cannot be completely excluded.

The evidence for afferent fibres to the nucleus of the horizontal limb of the diagonal band from the hypothalamus and/or mid-brain, and from the nucleus of the opposite side is very much stronger. After lesions which damage the medial forebrain bundle caudal to the nucleus of the diagonal band, degenerating fibres pass rostrally in the medial forebrain bundle, separate from and lateral to the fibres passing to the vertical limb of the diagonal band and the septum. Because these fragmenting fibres are found distributed *throughout* the nucleus and are not concentrated in those regions which coincide with the medial forebrain bundle, and because they are arranged in a definite 'pericellular' manner among the neurons of the nucleus, they are considered to terminate within the nucleus. Furthermore, there are noticeably fewer fibres in the medial forebrain bundle rostral to the nucleus than caudal to it. Ascending fibres from the midbrain and hypothalamus to the nucleus of the diagonal band have been reported previously by Guillery (1957), and by Nauta & Kuypers (1958), and the present findings are in close accord with their descriptions. It should be noted that these authors were predominantly concerned with the septal portion of the nucleus of the diagonal band, and the term 'horizontal limb of the diagonal band' does not occur in their accounts. The ascending hypothalamic connexions of the two limbs of the diagonal band would appear to differ in some respects, for a lesion in the rostral hypothalamus which is medial to the medial forebrain bundle causes degeneration in the nucleus of the vertical limb and in the septum, but not in the nucleus of the horizontal limb. Apart from this negative evidence that they do not arise in the medial hypothalamus, the precise site of origin of the afferent fibres to the nucleus of the horizontal limb of the diagonal band cannot be determined from these experiments. Some indication can be obtained from the experiments of Cowan, Guillery & Powell (1964) using the retrograde cell degeneration method. After lesions of the lateral pre-optic area, anterior to the hypothalamus and within the region of the horizontal limb of the diagonal band, cell shrinkage was found in the lateral hypothalamus, and especially in the supramamillary and ventral tegmental area.

Shute & Lewis (1967) have reported that the midbrain and hypothalamus are connected with the olfactory bulb through a possibly cholinergic fibre system. By the use of histochemical methods which stain acetylcholinesterase they were able to trace fibres from the ventral tegmental area and through probable relays in the lateral hypothalamic area, where there are many intensely staining cells, to the lateral pre-optic area; in this area another group of deeply staining cells was found, and from these cells axons which contain acetylcholinesterase enter the lateral olfactory tract and pass to the olfactory bulb. This system corresponds remarkably well with the afferent and efferent connexions of the nucleus of the horizontal limb of the diagonal band described here and in the previous paper, and together they provide a possible anatomical basis for the finding of Arduini & Moruzzi (1953) that stimulation of the midbrain can alter the activity of the olfactory bulb.

The pattern of degeneration found in the nucleus of the horizontal limb of the diagonal band after damage to the nucleus of the opposite side, or to the stria medullaris, is very similar to that present after a lesion in the hypothalamus. This commissural component of the stria medullaris has been described before (Cragg, 1961; Cowan *et al.* 1965) but its origin and termination have not been previously related to the nucleus of the horizontal limb of the diagonal band. That they arise in this nucleus is indicated by the same experiments which were used to determine the origin of the centrifugal fibres to the olfactory bulb; lesions which damage the nucleus produce degeneration in the contra-lateral stria medullaris and nucleus of the horizontal limb of the diagonal band, but lesions anterior to the nucleus, or caudal to the stria medullaris, do not. This finding of commissural connexions between the two nuclei of the horizontal limb of the diagonal band indicates that the two structures in the basal forebrain which are known to send fibres direct to the olfactory bulb, the nucleus of the horizontal limb of the diagonal band and the anterior olfactory nucleus, are both connected across the mid-line with their counterpart on the opposite side. Furthermore, there is evidence to suggest that in both structures the ipsilaterally and contralaterally directed axons are branches from the same cells (Valverde, 1965; Price & Powell, 1970*a*).

As well as the commissural connexions, fibres have been described which ascend in the habenulo-peduncular tract and then pass rostrally through the stria medullaris to the lateral pre-optic area and substantia innominata of the same side (Cragg, 1961; Mitchell, 1960) and it is possible, therefore, that these provide an additional pathway for fibres from the hypothalamus to the nucleus of the horizontal limb of the diagonal band. Afferent fibres may also come from the medio-dorsal nucleus of the thalamus, and from that part of the frontal cortex to which the medio-dorsal nucleus projects. Although any experimental approach to the medio-dorsal nucleus is complicated by the close proximity of the stria medullaris, Nauta (1962) has placed lesions in this nucleus in the monkey by inserting the electrode at an angle in order to avoid the stria. He reports that degenerating fibres pass from the magno-cellular division of the medio-dorsal nucleus to the nucleus of the diagonal band, but in his figures the degeneration does not appear to be present throughout the entire nucleus, and might again be due to the many fibres passing through the region. Similarly, Leonard (1968) has recently reported that the portions of the frontal cortex of the rat which receive a projection from the medio-dorsal nucleus send fibres into the lateral pre-optic area, although she does not relate this to the nucleus of the diagonal band. A final conclusion on whether the medio-dorsal nucleus or its related cortex send fibres to this nucleus must, therefore, await further investigation, but such projections might be expected in view of the strong projection from the pyri-form cortex to the medio-dorsal nucleus (Powell *et al.* 1963, 1965).

Any centrifugal system in a sensory pathway would be expected to receive direct or indirect 'information' from the more peripheral structures which it is influencing. On *a priori* grounds, therefore, it is surprising to find that there are no direct connexions from the olfactory bulb or one of its primary projection sites; originally it was expected that there would be a 'feed-back loop' consisting of the olfactory bulb, the pyriform cortex, amygdala, or olfactory tubercle, and the nucleus of the horizontal limb of the diagonal band. Our experimental evidence indicates, however, that the only afferent connexions of this nucleus are from the hypothalamus and mid-brain, and from the nucleus of the opposite side. As the pyriform cortex, amygdala and olfactory tubercle send strong fibre projections into the hypothalamus (cf. Powell *et al.* 1963, 1965) it is very probable that olfactory information does reach the nucleus of origin of the olfactory centrifugal fibres, but only after convergence of these different pathways upon the hypothalamus and interaction with midbrain and hypothalamic influences. There are, however, several other sets of connexions which could be described as 'loops' through which activity in the mitral cells of the olfactory bulb may be modified (Price, 1969); these are confined to the olfactory system, and they increase steadily in the degree of integration which may occur within them. In all, five different, although overlapping, systems can be outlined with some degree of confidence at the present time (Fig. 10); there may be others which have not yet been completely elucidated.

(1) The most immediate of these is the 'reciprocal synapse' which has been found between mitral cell dendrites and the spine-like 'gemmules' of the peripheral pro-cesses of the granule cells of the olfactory bulb (Hirata, 1964; Andres, 1965; Rall, Shepherd, Reese & Brightman, 1966). This unusual synapse is orientated both from mitral to granule cell, and from granule to mitral cell, and may provide a mechanism for the modification of a stream of impulses within an individual mitral cell, or through spread of activity in the branching granule cell processes, in immediately neighbouring mitral cells. There is some evidence that the mitral to granule cell half of this synapse is excitory and that the granule to mitral cell half is inhibitory (Rall *et al.* 1966; Price, 1968).

(2) The next stage involves one further synapse, and probably acts on mitral cells some distance from the cell which was initially active, through the recurrent col-laterals of the mitral cell axons. These collaterals are given off in the internal plexi-form layer immediately deep to the mitral cell bodies and ramify in the granule cell layer and the external plexiform layer (Cajal, 1911); they probably have an extensive

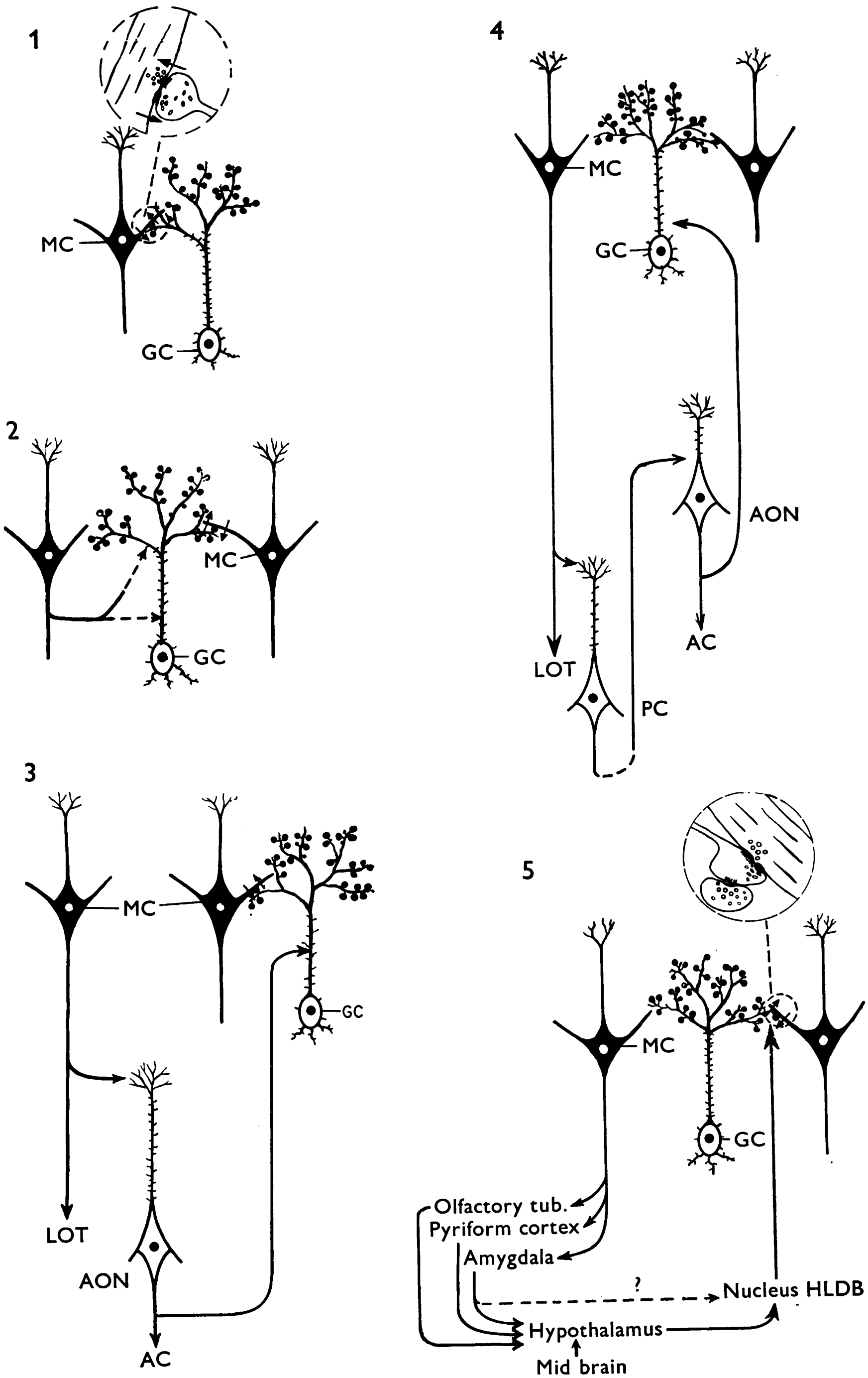


Fig. 10. Schematic figure to show mitral cell-granule cell inter-relationships and the pathways by which progressively more central parts of the olfactory pathway may reciprocally influence the mitral cell. (1) The reciprocal synapse between mitral cell dendrite and gemmule of granule cell. (2) Collateral of mitral cell axon to granule cell. (3) Collateral of mitral cell axon to neuron of anterior olfactory nucleus and collateral of axon of latter cell to granule cell. (4) Mitral cell axon to neuron of pyriform cortex and association fibre pathway from this cortical area to anterior olfactory nucleus and in turn to granule cell. (5) Projection of mitral cell to olfactory tubercle, pyriform cortex and amygdala, and pathway from these to hypothalamus and thence to the nucleus of the horizontal limb of the diagonal band. From this nucleus centrifugal fibres pass rostrally to end upon gemmules of granule cell.

distribution, as small lesions in the superficial layers of the olfactory bulb produce intense degeneration in the internal plexiform layer of large portions of the bulb (Lohman & Mentink, 1969; our own unpublished observations). They almost certainly terminate upon the spines and 'gemmules' of the granule cell layer (Price & Powell, 1970*b*). The third and fourth 'loops' involve the cortical structures caudal to the olfactory bulb, the anterior olfactory nucleus and the pyriform cortex.

(3) Axons in the lateral olfactory tract give collaterals into the anterior olfactory nucleus which terminate upon the superficial half of the apical dendrites of the pyramidal cells (Cajal, 1911; White, 1965; Heimer, 1968) and according to Valverde (1965) these same neurons, as well as sending axons to the opposite side through the anterior commissure, have collaterals which turn anteriorly and enter the ipsilateral olfactory bulb. Within the bulb these collaterals have been found to terminate upon the spines of the peripheral processes in the granule cell layer, and probably upon 'gemmules' of the same processes in the external plexiform layer (Price & Powell, 1970*b*).

(4) The axons of the lateral olfactory tract also give off collaterals which terminate on the superficial portions of the pyramidal cells of the pyriform cortex (White, 1965; Heimer, 1968) and the pyriform cortex in turn sends fibres rostrally to the anterior olfactory nucleus, where they terminate on the deeper portions of the pyramidal cell apical dendrites (Heimer, 1968; Price & Powell, 1970*a*). For simplicity the figure which illustrates this 'loop' has been drawn as if the same cells of the pyriform cortex which receive the olfactory fibres also project to the anterior olfactory nucleus, but it should be emphasized that this need not be so, and that these corticofugal fibres could arise from the deeper cells of the cortex. This 'loop' includes at least four synapses, and possibly more. It must also be noted that both 'loops' which pass through the anterior olfactory nucleus may be more complex than has been outlined here and may have to be revised as more becomes known about this cortical structure. It is already clear that the anterior olfactory nucleus should not be thought of simply as a relay station (Lohman, 1963).

(5) The last set of connexions consists of the centrifugal fibres to the olfactory bulb from the nucleus of the horizontal limb of the diagonal band described in the previous paper (Price & Powell, 1970*a*) and the afferent connexions of this nucleus reported here. The centrifugal fibres have been shown to synapse with the gemmules on the granule cell peripheral processes in the external plexiform layer, in very close relation to the reciprocal synapses (Price, 1968). This system is obviously not a simple 'feedback loop', as there must be interactions within and among the pyriform cortex, amygdala and olfactory tubercle, as well as further modification of olfactory 'information' by the hypothalamus. It would probably be more correct to consider this centrifugal system as a mechanism by which the midbrain and hypothalamus can influence the olfactory system. The 'centrifugal' system therefore stands apart from the other four which have been given here; the latter are really one system, made of progressively extending loops, but contained within the part of the brain primarily concerned with olfaction. The 'centrifugal' system, acting through the nucleus of the horizontal limb of the diagonal band, relates the non-olfactory part of the brain to the olfactory system, and does so at a very peripheral level.

Whether this pattern of *two* types of increasingly complex 'loops' of connexions, through which peripheral cells of a sensory pathway may be influenced, is common to all sensory pathways, and whether the capacity to link an individual sensory pathway with other parts of the brain is the feature in all sensory pathways which distinguishes 'centrifugal' fibre systems from the reciprocal fibre systems which connect only structures within a particular sensory pathway must remain speculative until further evidence is available. However, examples of each of these two types of 'loops' are present in other sensory pathways. The cortico-thalamic fibres from each of the sensory areas of the neocortex to the related sensory relay nuclei (Jones & Powell, 1969) and the fibres from the somatic sensory cortex to the dorsal column nuclei (Kuypers & Tuerk, 1964) belong to the type of 'loop' in which the connexions are between parts of one sensory pathway only, while the centrifugal fibre system found within the avian visual pathway is strikingly similar in many respects to the centrifugal 'loop' of the olfactory pathway. Like the centrifugal fibres to the olfactory bulb, the centrifugal fibres to the retina arise from a discrete nucleus in the midbrain and this nucleus receives afferent fibres from a structure, the tectum (McGill, Powell & Cowan, 1966), in which there is the possibility of integration of 'information' from peripheral parts of the visual system with that from other sensory systems. In addition, in both the olfactory and visual pathways the centrifugal fibres end upon an interneuron which does not have a typical axonal process, which is related to the second-order neuron of the pathway, and which takes part in a reciprocal synapse (cf. Price, 1968; Dowling & Cowan, 1965; Dowling & Boycott, 1966).

SUMMARY

The afferent connexions of the nucleus of the horizontal limb of the diagonal band, which is the site of origin of the centrifugal fibres to the olfactory bulb, have been studied in the rat using the methods of Nauta & Gygax (1954) and of Fink & Heimer (1967).

Two groups of afferent fibres to this nucleus have been found. The first group comes from regions caudal to the nucleus, from the hypothalamus and possibly from the midbrain, and these fibres pass forwards in the medial forebrain bundle, lateral to the fibres to the vertical limb of the diagonal band. The second group is a commissural projection, passing through the stria medullaris and habenular commissure, from the same nucleus of the opposite side.

The pyriform cortex, amygdala and olfactory tubercle send fibres into the medial forebrain bundle in very close relationship with the nucleus, but the appearance and distribution of the degeneration after lesions of these structures suggest that these fibres do not terminate within the nucleus.

The present evidence, taken in conjunction with other findings on the organization of the olfactory pathway, indicate that there are at least five different, though overlapping 'loops' of connexions through which activity in the mitral cells of the olfactory bulb may be modified.

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