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The origin of the mamillary peduncle and other hypothalamic connexions from the midbrain

By W. M. COWAN, R. W. GUILLERY and T. P. S. POWELL
Departments of Human Anatomy, Oxford, and University College London

INTRODUCTION

Recent experimental work has shown that there are three ascending pathways which link the midbrain to the hypothalamus: the mamillary peduncle, the medial forebrain bundle and the periventricular fibre system. From the region of the dorsal and deep tegmental nuclei the fibres of the mamillary peduncle run ventrolaterally through the midbrain tegmentum and pass on either side of the medial lemniscus to reach the ventral aspect of the midbrain. They then pass rostrally through the rootlets of the third nerve, immediately lateral to the interpeduncular nucleus. The peduncle ends as a compact bundle dorsal to the lateral mamillary nucleus, sending most of its fibres into the medial and lateral mamillary nuclei, but some continue rostrally with the medial forebrain bundle into the lateral supramamillary region and lateral hypothalamus, and a few reach as far as the septum. Caudally the medial forebrain bundle itself is first recognized in the ventral tegmental area from which it can be traced forwards, just dorsal to the mamillary peduncle, and thence through the lateral supramamillary, lateral hypothalamic and preoptic regions to the septum and olfactory tubercle. The periventricular fibres enter the hypothalamus from the periaqueductal region of the midbrain. At posterior hypothalamic levels many join the hypothalamic periventricular system, but a large number of fibres sweep ventrally and laterally to join the medial forebrain bundle in the lateral hypothalamus (Fox, 1941; Guillery, 1957; Nauta & Kuypers, 1958; Morest, 1961).

With regard to the origin of these pathways Quensel (1911), Fox (1941) and Akert & Andy (1955), using the retrograde cell degeneration method, have shown that the mamillary peduncle arises, in part at least, from the deep tegmental nucleus. In addition Akert & Andy have shown that some of the fibres of the mamillary peduncle arise in the dorsal tegmental nucleus, and this has been confirmed by Guillery (1956) and Morest (1961) using the Nauta method. An even more extensive origin from other nuclei of the mesencephalic tegmentum such as the ventral tegmental area (including the so-called nucleus of the mamillary peduncle), the tegmental reticular nucleus, the nucleus centralis superior and the periaqueductal grey has been suggested (Papez, 1932; Fox, 1941; Nauta & Kuypers, 1958; Morest, 1961). The origins of the other two fibre systems are less clear, and in particular it is not known to what extent the areas which have been implicated in the origin of the mamillary peduncle may also contribute fibres to the medial forebrain bundle and the periventricular fibre system. The studies which have been done on these two systems have been based on fibre degeneration techniques which, in general, are less suitable for delimiting the site of origin of a pathway than the retrograde cell degeneration method where this technique yields a positive result.

The present investigation was undertaken to determine to what extent these three bundles arise from separate nuclear groups in the mesencephalon, and, by placing a variety of lesions in the hypothalamus and rostral midbrain of rats and rabbits, to study the relationship between them. One of the most significant findings to emerge, however, is the observation that the degenerative reaction of the cells contributing to these three systems varies markedly in different nuclei and so raises several problems regarding the interpretation of cellular degeneration in the central nervous system

MATERIAL AND METHODS

The material used for this study consists of the brains of twelve rabbits and eighteen rats. In all the rabbits electrolytic lesions were placed in the rostral midbrain or hypothalamus with the aid of a stereotaxic instrument, and in the rats similar lesions were placed in these areas, either through a dorsal approach, or by way of the parapharyngeal approach of Ingle & Griffith (1942). The animals were allowed to survive for periods of 1–3 months after which the brains were fixed in a mixture of 70 % alcohol and 2 % acetic acid, dehydrated, and embedded in paraffin. A 1 in 5 series of 25 μ coronal sections through the forebrain and midbrain was routinely mounted and stained with thionin, and, where necessary, additional sections were similarly treated.

In view of the different terminologies that have been used in previous publications, both for the subdivisions of the mamillary nuclei and for the nuclei of the brainstem, it is necessary to define the terms which are employed in the present paper. For the mamillary nuclei of the rat we have followed Gurdjian's nomenclature (1927); in general, the subdivisions of these nuclei are similar in the rabbit and the rat, and have been homologized on the basis of their efferent connexions (Cowan & Powell, 1955). For the nuclei of the rat mesencephalon Gillilan's classification (1943) has been adopted with certain modifications. The nucleus mesencephalicus profundus (nucleus tegmenti of Gudden) will be called the deep tegmental nucleus. It is a clearly defined group of relatively large cells lying immediately caudal to the decussation of the superior cerebellar peduncle. Rostral to it, and just ventral to the caudal part of the decussation is a group of smaller cells which is separated from the main part of the deep tegmental nucleus by a short interval. These smaller cells lie adjacent to the nucleus centralis superior, but since their retrograde reactions are identical to those of the deep tegmental nucleus they will be grouped with that nucleus in the following account. The dorsal tegmental nucleus has been divided, on morphological grounds and on the basis of the retrograde cell degeneration that follows interruption of the mamillary peduncle, into four parts: central, ventromedial, anterior and posterior (see also Morest, 1961). The pars centralis is the largest of these subdivisions and forms a distinct oval group of small and medium-sized cells. The latter are more deeply staining and are concentrated in the ventral part of this subdivision of the nucleus. The pars ventromedialis is for the most part composed of medium-sized cells scattered along the ventromedial aspect of the pars centralis and of the pars posterior and lies close to the dorsal aspect of the medial longitudinal bundle. Both the pars anterior and the pars posterior are composed of small cells and form, respectively, rostral and caudal extensions of the pars centralis.

In the rabbit the dorsal and deep tegmental nuclei closely resemble those of the rat. The deep tegmental nucleus again has a small-celled rostral part and, in the pars centralis of the dorsal tegmental nucleus, the larger cells are similarly grouped in the ventral part of the nucleus (Pl. 1, fig. 1). The pars ventromedialis is separated caudally from the caudal part of the dorsal nucleus of the raphe by an unnamed group of small cells which has never shown signs of retrograde degeneration after lesions of the mamillary peduncle. The partes anterior and posterior are again present in the rabbit, but are less clearly defined than in the rat. One nucleus not referred to by Gillilan is the tegmental reticular nucleus of Bechterew; this has been labelled 'nucleus papilioformis' by Meessen & Olszewski (1949).

RESULTS

The experiments to be described indicate that ascending fibres pass to the hypothalamus from four principal sites in the midbrain: the deep and dorsal tegmental nuclei, the ventral tegmental area, and the tegmental reticular nucleus of Bechterew. The evidence bearing on the projection of each of these areas will be presented separately.

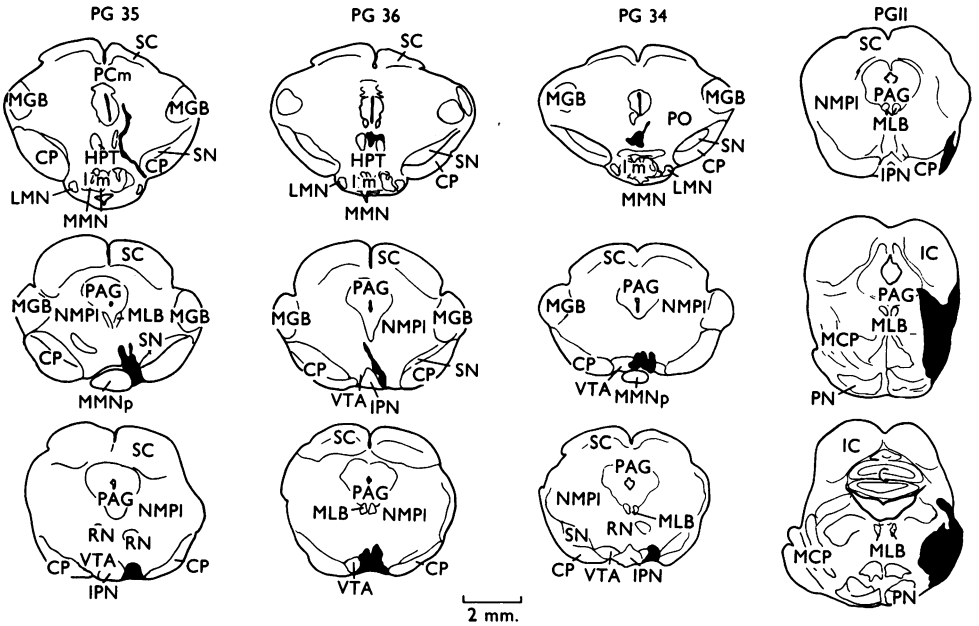
The dorsal and deep tegmental nuclei

The rat experimental material can be divided, on the basis of the position of the lesion, into three groups. In the first group the lesion has involved the mamillary peduncle behind the mamillary bodies. In the second group the peduncle has been spared except for its mamillary ending. In the third group the lesion lies entirely rostral to the mamillary bodies, involving the medial forebrain bundle in the hypothalamus or septum. Since the course of the mamillary peduncle has already been described, and since the present section is only concerned with cellular changes occurring in the dorsal and deep tegmental nuclei following interruption of the mamillary peduncle, a full description of the lesions will not be given. The site and extent of most of the lesions are shown in the accompanying text-figures, and others are given in more detail in subsequent sections.

(*a*) *Lesions behind the mamillary bodies.* Experiment PG 35 is typical of the first group. In this experiment the lesion passes ventrally through the posterior part of the thalamus and through the left supramamillary region, where it has interrupted the mamillotegmental tract of that side. The lesion ends in the ventral tegmental area (of Tsai), where it has completely divided the mamillary peduncle (Text-fig. 1). The main part of the lesion does not extend caudal to the interpeduncular nucleus, nor does it cross the midline. However, the ventral part of the periaqueductal grey just rostral to the third nerve nuclei has undergone bilateral necrosis with severe glial reaction.

The dorsal and deep tegmental nuclei both show well-marked cellular changes on the side of the damaged mamillary peduncle. The deep tegmental nucleus has undergone complete cell loss and subsequent gliosis in all but its posteromedial part, where a few shrunken cells persist. In the dorsal tegmental nucleus the pars ventromedialis shows marked cell shrinkage but no clear evidence of cell loss, and a similar change is seen in the pars centralis, especially among the larger cells in the ventral part of the nucleus. The cells in the anterior and posterior parts of the nucleus show no change.

In experiment PG 34 the main part of the lesions lies in the supramamillary region and in the mamillary peduncle of the left side immediately behind the mamillary bodies (Text-fig. 1). Just caudal to the mamillary bodies the lesion also encroaches upon the medial aspect of the right mamillary peduncle. On the left side the dorsal and deep tegmental nuclei show the same changes as in PG 35. On the right side the dorsal tegmental nucleus appears to be unaffected but the deep tegmental nucleus shows marked cell loss in its caudal part; in the rostral third of the



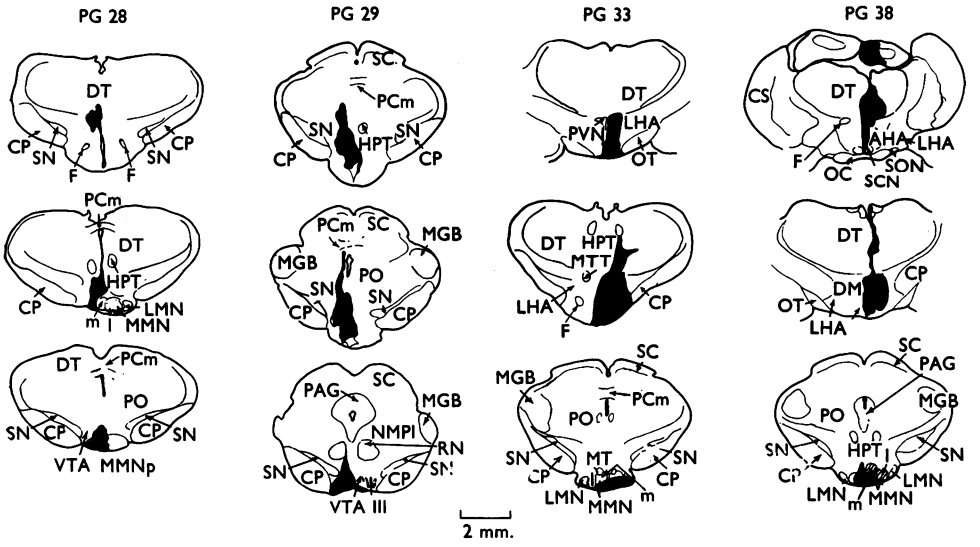
Text-fig. 1. Diagrams to show the lesions in the first group of rat experiments in which the mamillary peduncle has been interrupted behind the mamillary nuclei. In this and in the subsequent text-figures the lesion is shown in black at three representative levels throughout its antero-posterior extent. All the diagrams have been prepared from projected outlines of sections from the relevant brain.

nucleus an appreciable number of cells remain. This experiment, therefore, substantiates the conclusion that the origin of the mamillary peduncle is from the dorsal and deep tegmental nuclei, and in addition suggests that the fibres which arise in the deep tegmental nucleus pass forward in the medial part of the peduncle while those which occupy the lateral part of the peduncle at the level of the interpeduncular nucleus have their origin in the dorsal tegmental nucleus. Experiment PG 36 in which the relevant part of the lesion (Text-fig. 1) is identical with that of PG 34 also serves to confirm this conclusion.

The lesion in experiment PG 11 is situated much further caudally in the midbrain and is strictly unilateral (Text-fig. 1). The main part of the lesion lies caudal to the level of the interpeduncular nucleus and includes the lateral quarter of the medial lemniscus and those fibres of the mamillary peduncle that pass lateral to the lemniscus. There is marked cell shrinkage and possibly some cell loss in the deep

tegmental nucleus of this side and in the dorsal tegmental nucleus there is definite cell shrinkage in the pars ventromedialis, but the pars centralis is unaffected. From this it would appear that the medio-lateral organization of the fibres that is found in the mamillary peduncle just behind the mamillary bodies is not present at more caudal levels.

(*b*) *Lesions involving the mamillary bodies.* In experiment PG 29 the lesion is again bilateral. On the right side the mamillary nuclei and the ventral tegmental area



Text-fig. 2. Diagrams to show the lesions in four of the rat experiments of group 2. In these experiments the mamillary nuclei have been damaged, but the mesencephalic course of the mamillary peduncle has been spared.

rostral to the emergence of the third nerve have been completely destroyed, but on the left the pars medialis, the medial margin of the pars lateralis and the medial two-thirds of the pars posterior of the medial mamillary nucleus are damaged. The lateral mamillary nucleus of this side is spared. The lesion on the right side is thus typical of the first group of experiments, and the degeneration is exactly comparable to that seen in PG 35; the left side may be regarded as the first example of the second group since there is no evidence in any of this material for a crossed component in the mamillary peduncle. On the left the deep tegmental nucleus shows appreciable cell loss, but there are a number of pale staining cells remaining throughout the nucleus. There is no evidence of degeneration in the dorsal tegmental nucleus but in the absence of a normal side for comparison slight changes could easily be missed.

A comparison of the two sides in this experiment suggests that the deep tegmental nucleus projects to the medial mamillary nucleus while the dorsal tegmental nucleus is related to the lateral mamillary nucleus or the medial forebrain bundle. The remaining animals of the second group provide further evidence about the termination of these components.

In experiment PG 28 the damage to the mamillary nuclei is greater than in the

other animals of this group. The main part of the lesion lies dorsal and rostral to the mamillary nuclei but caudally it involves the mamillary region bilaterally. The lateral supramamillary region and the rostral end of the mamillary peduncle are spared on both sides. The right medial mamillary nucleus has been completely destroyed and the lateral mamillary nucleus of this side has disappeared completely. In view of the distortion in this region it is impossible to determine whether the nucleus was included in the primary lesion or whether its cells have undergone complete retrograde degeneration. On the left side only the caudal half of the pars medialis and the medial third of the pars posterior of the medial mamillary nucleus have been injured; the lateral mamillary nucleus is quite unaffected on this side (Text-fig. 2).

The deep tegmental nucleus of the right side shows complete cell loss and the pars centralis of the dorsal tegmental nucleus shows the characteristic cell shrinkage, especially in the larger cells of its ventral portion (Pl. 2, fig. 3). The pars ventromedialis shows some cell shrinkage but this is definitely less marked than the shrinkage seen after complete division of the mamillary peduncle. On the left side there is no clear evidence of any cell change in either of the tegmental nuclei, but again, in the absence of a control side minor changes cannot be excluded. Since in this experiment the changes in the pars ventromedialis of the right are less marked than after complete division of the peduncle it would appear that this subdivision of the dorsal tegmental nucleus does not project primarily to the medial mamillary nucleus; whether it projects into the medial forebrain bundle, or to the lateral mamillary nucleus remains to be determined.

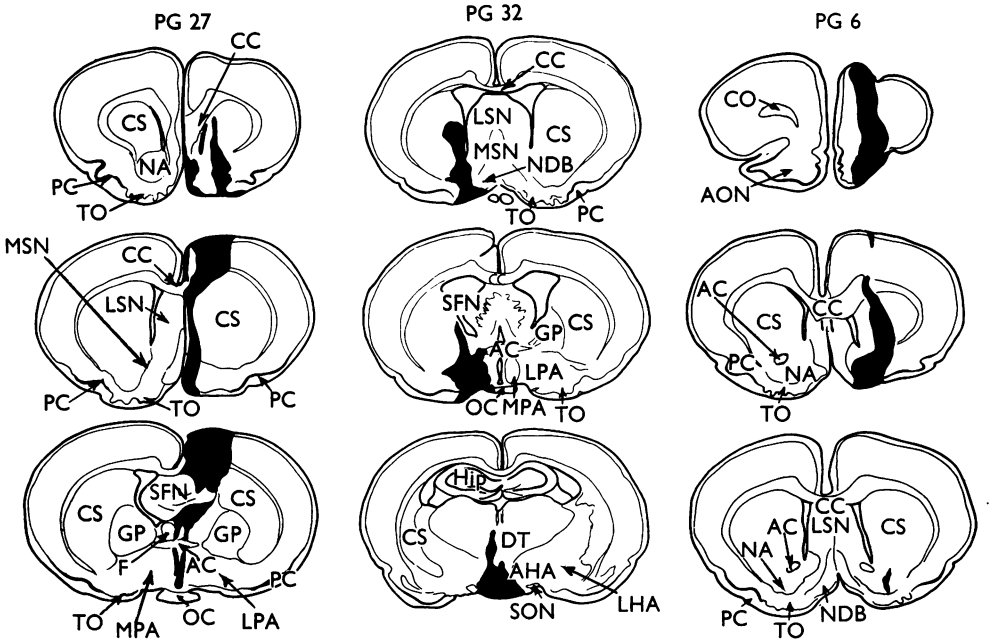
Experiment PG 38 throws further light on the projection of the pars ventromedialis of the dorsal tegmental nucleus. In this experiment a large lesion has destroyed the medial third of the rostral half of the thalamus and the whole of the hypothalamus of the left side, between the level of the optic chiasma and the ventromedial hypothalamic nucleus. The mamillary part of the lesion is a narrow caudal extension which passes through the premamillary nuclei of the right side and into the medial mamillary nucleus of this side. Here it has destroyed the rostral half of the pars medialis and the medial two-thirds of the pars lateralis, but has spared the pars posterior and the lateral mamillary nucleus. There has been no direct involvement of the mamillary nuclei of the left side, but there are extensive retrograde changes in these nuclei following the interruption of the principal mamillary tract (Text-fig. 2).

On the right side the deep tegmental nucleus shows a partial cell loss and, more strikingly, shrinkage and pallor of the surviving cells. The dorsal tegmental nucleus shows no change on this side but in the pars ventromedialis on the left side there are slight, but definite, signs of cell shrinkage. The degeneration in the right deep tegmental nucleus is clearly related to the lesion in the rostral part of the medial mamillary nucleus of that side, but as there has been no direct involvement of the mamillary nuclei of the left side the degeneration in the left pars ventromedialis must be interpreted a secondary either to the retrograde degeneration in the lateral mamillary nucleus or to the lesion in the premamillary part of the medial forebrain bundle.

In experiment PG 39 the premamillary part of the lesion includes much of the periventricular region of the diencephalon but spares the medial forebrain bundle on

both sides. In addition there is a small caudal incision that passes through the rostral part of the left medial mamillary nucleus and damages the adjoining parts of the pars lateralis and the pars medialis. The only change that can be seen in the tegmental nuclei is slight cell shrinkage and pallor in the left deep tegmental nucleus.

In PG 33 the lesion has partially destroyed the mamillary nuclei of both sides in addition to severe bilateral involvement of the medial parts of the hypothalamus. On the left side the most rostral part of the mamillary region has been completely

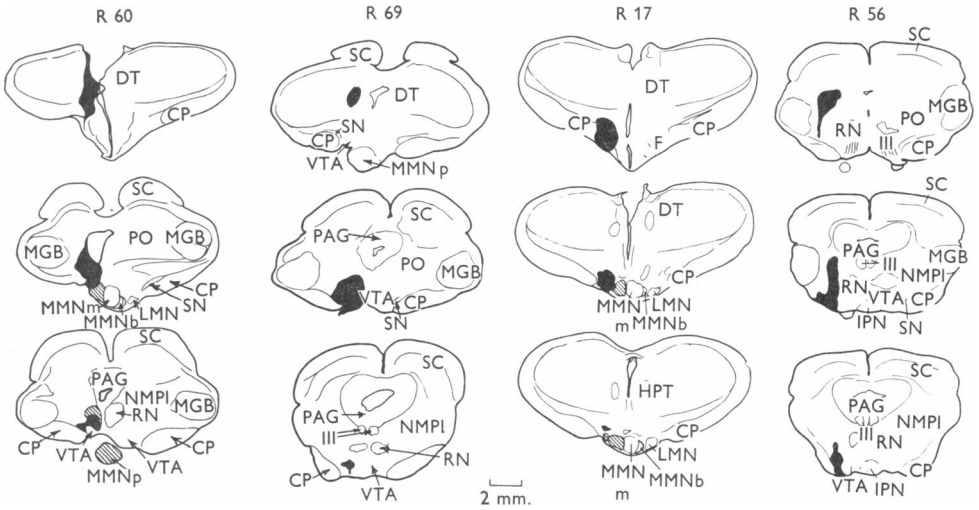


Text-fig. 3. Diagrams of the lesions relevant to the cellular degeneration in the pars ventromedialis of the dorsal tegmental nucleus and in the ventral tegmental area.

destroyed together with the principal mamillary tract and the greater part of the lateral mamillary nucleus. However, the mamillary peduncle as it lies dorsal to the lateral mamillary nucleus has been spared. The posterodorsal part of the medial mamillary nucleus has not been directly damaged but shows extensive retrograde degeneration. On the right side only the ventral third of the pars medialis and of the pars lateralis of the medial mamillary nucleus have been damaged. Rostral to the mamillary bodies the medial forebrain bundle has been interrupted on the left but not on the right side (Text-fig. 2). On the right side there is no clear evidence of any cell change in the tegmental nuclei. On the left the pars centralis and the pars ventromedialis of the dorsal tegmental nucleus show well-marked cell shrinkage, but the changes in the pars centralis are less severe than after complete division of the mamillary peduncle. The deep tegmental nucleus on this side shows a partial cell loss with shrinkage and pallor in many of the remaining cells, especially in the posterior part of the nucleus.

(*c*) *Lesions rostral to the mamillary bodies.* Some of the experiments in which the

premamillary medial forebrain bundle has been damaged also show degeneration in the pars ventromedialis of the dorsal tegmental nucleus. None of these experiments shows degeneration in any other part of the tegmental nuclei. In experiment PG 27 the lesion has destroyed practically the whole of the precommissural septum on the left side (Text-fig. 3). It stops sharply at the midline and includes only a small medial strip of the caudate nucleus. The pars ventromedialis of the dorsal tegmental nucleus of this side shows definite cell shrinkage, which, however, is not as marked as



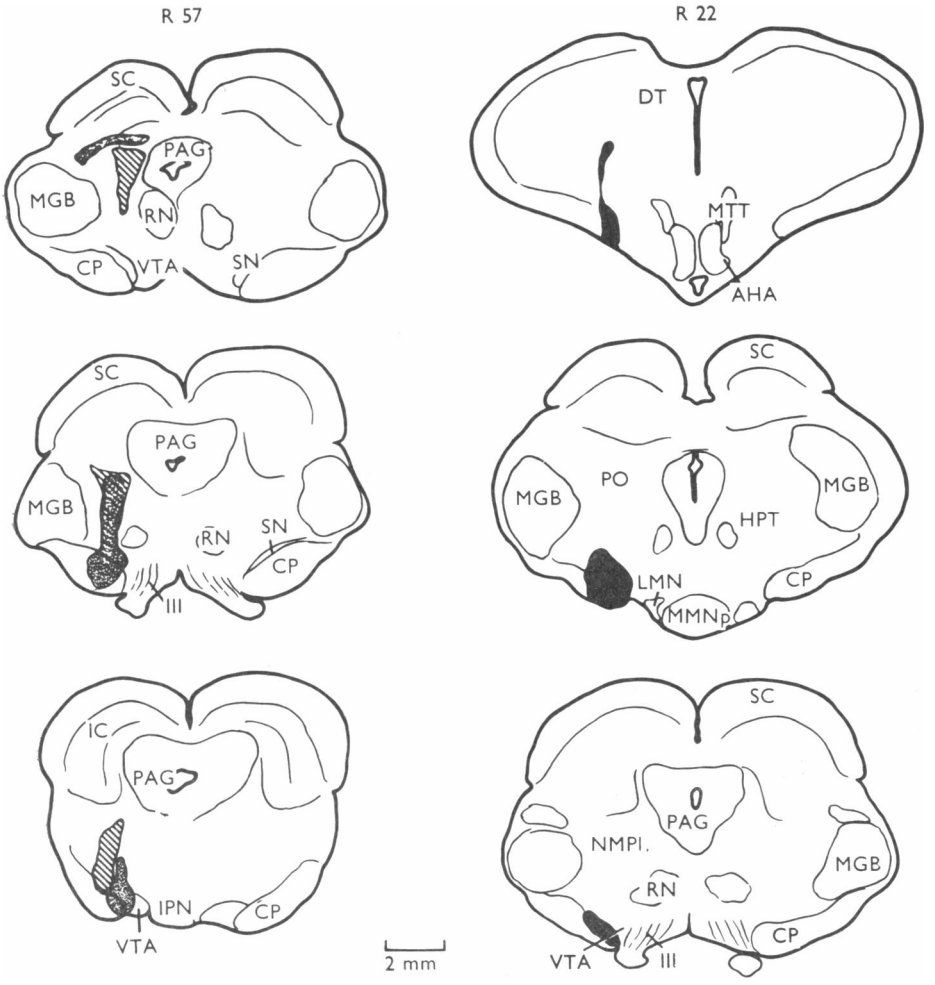
Text-fig. 4. Diagrams to show the lesions in four of the rabbit experiments in which degeneration is found in the dorsal and deep tegmental nuclei. Hatched areas indicate nuclei in which marked retrograde cell degeneration has occurred.

after complete destruction of the mamillary peduncle. In PG 32 (Text-fig. 3) the lateral and medial preoptic areas of the right side are destroyed together with the medial hypothalamic areas of both sides back to the level of the ventromedial hypothalamic nucleus. There is no change in the tegmental nuclei apart from some cell shrinkage in the pars ventromedialis of the right side. The degeneration in the pars ventromedialis shows no correlation with retrograde degeneration in the mamillary nuclei (see p. 350), but neither does it show an absolute correlation with damage to the medial forebrain bundle, since two experiments (PG 30 and 31) failed to show any change in the dorsal tegmental nucleus after lesions that included a considerable part of the medial forebrain bundle at preoptic levels. Maximal degeneration can only be seen in the pars ventromedialis after lesions that include both the medial forebrain bundle and the lateral mamillary components of the mamillary peduncle.

Since the rabbit experiments only differ in minor points from the rat material they will be described more briefly. In experiment R 60 there is an extensive unilateral lesion in the medial part of the thalamus and hypothalamus which ends caudally in the most rostral part of the ventral tegmental area, where the mamillary peduncle has been interrupted (Text-fig. 4). The deep tegmental nucleus on this side shows complete cell loss with gliosis. In the pars centralis of the dorsal tegmental nucleus

there is moderately severe cell shrinkage and the pars ventromedialis shows definite evidence of cell loss with shrinkage and pallor of the remaining cells.

In experiments R 69, R 54 and R 17 the mamillary peduncle is again destroyed unilaterally either caudal to the mamillary bodies or at its termination within the mamillary bodies (Text-fig. 4). The degenerative changes in the dorsal and deep tegmental nuclei do not differ significantly from those in R 60 (cf. Pl. 1, figs. 1 and 2).



Text-fig. 5. Diagrams of the lesions in two of the rabbit brains, R 57 and R 22, in which the mamillary peduncle escaped injury. In the diagram of R 57 the lesion, which is shown by hatching, has superimposed upon it a tracing of the lesion in R 56 (stippled). Observe that in R 57 the ventral tegmental area has not been involved.

Some of the lesions (R 56, R 27 and R 23) have produced partial destruction of the mamillary peduncle in the midbrain. These all show some degeneration in the dorsal and deep tegmental nuclei but, unlike the rat material, they give no clear indication for an organization in the peduncle. Three other brains are of interest since in each

of these the lesion has spared the mamillary peduncle (lying immediately lateral to it at the level of the ventral tegmental area) while damaging those areas that have been incidentally destroyed in the preceding experiments. The absence of degenerative changes in the dorsal and deep tegmental nuclei in these brains may therefore be regarded as suitable control material for the findings in the other experiments. In Text-figure 5 the lesion in the first of these experiments, R 57, is compared with that in R 56. While R 56, in which the mamillary peduncle has been damaged but the mamillotegmental tract spared, shows marked cell shrinkage in the pars centralis of the dorsal tegmental nucleus and considerable cell loss in the deep tegmental nucleus, R 57 shows no change in either of these nuclei. The figure shows that the two lesions are similar in position and extent, only differing in that there is no involvement of the mamillary peduncle and ventral tegmental area in R 57. The damage in the region of the ventral tegmental area and mamillary peduncle must therefore be responsible for the degeneration in the tegmental nuclei in R 56.

The lesions in the other control experiments, R 23 and R 22 are similar, and as R 22 has the larger lesion only this brain will be described. In this experiment the lesion begins rostrally as a small area of destruction in the medial part of the cerebral peduncle at about the level of the ventromedial hypothalamic nucleus. The lesion increases in size as it is traced caudally and comes to include the lateral part of the lateral hypothalamic area. At the level of the mamillary nuclei it lies immediately dorsolateral to the lateral mamillary nucleus without damaging any part of the mamillary complex itself. Behind the mamillary nuclei the area of destruction is immediately lateral to the ventral tegmental area and mamillary peduncle and continues in this position to the level of the interpeduncular nucleus, where it ends (Text-fig. 5). Again, there is no evidence of cellular degeneration in either the dorsal or the deep tegmental nucleus.

The ventral tegmental area

The rat experiments which show degeneration in the ventral tegmental area can be divided into two groups: first, those in which the caudal part of the lesion has involved the mamillary bodies or the supramamillary region, and secondly, experiments in which the premamillary medial forebrain bundle has been involved in some part of its course without any involvement of the mamillary nuclei. Only three experiments will be considered in the first group: PG 28, PG 33 and PG 38.

In experiment PG 33 the medial hypothalamus has been damaged bilaterally at the level of the anterior hypothalamic area, but further caudally the lesion moves to the left and comes to include most of the medial forebrain bundle of that side back to the level of the mamillary bodies. The mamillary lesion (which has been described in detail above) includes the mamillary nuclei themselves but spares most of the supramamillary region bilaterally (Text-fig. 2). The ventral tegmental area on the left side shows marked cell shrinkage and gliosis but on the right side it is quite unaffected. In PG 28 the relevant part of the lesion lies in the medial supramamillary region of both sides, but extends further laterally on the right than on the left (Text-fig. 2). There is cell shrinkage in the right ventral tegmental area but not on the left, which suggests that the majority of the fibres that ascend from the ventral tegmental area pass through the lateral rather than the medial supramamillary

region. Experiment PG 38 has a lesion in the left medial forebrain bundle at anterior hypothalamic levels (Text-fig. 2). Further caudally the lesion moves to the right to include the periventricular region at the level of the ventromedial hypothalamic nuclei, and finally comes to end in the right medial supramamillary region and medial mamillary nucleus. The ventral tegmental area shows no evidence of cell change on the right side, but there is distinct cell shrinkage and gliosis on the left. A comparison of the two sides in this experiment confirms that most of the fibres from the ventral tegmental area pass through the lateral supramamillary region and also indicates that many of these fibres pass rostrally in the medial forebrain bundle for considerable distances.

In the first three experiments of the second group, PG 25, PG 30 and PG 31, the medial forebrain bundle has been damaged in the region of the lateral preoptic area or at the level of the diagonal band. In each there is a distinct cell shrinkage in the homolateral supramamillary region and in the ventral tegmental area. There is also some evidence for slight cell shrinkage throughout the lateral hypothalamic area, but this is much less marked than in the supramamillary and ventral tegmental areas.

Three other rat experiments are of interest in this group. In PG 32 the medial forebrain bundle has been damaged rostral to, and at the level of, the anterior hypothalamic area on the right side. Behind this level the lesion has crossed the midline to include the medial, but not the lateral part of the rostral hypothalamus on the left (Text-fig. 3). On the right there is some cell shrinkage throughout the lateral hypothalamic area which becomes appreciably more marked as the sections are traced caudally into the supramamillary region and ventral tegmental area (Pl. 2, fig. 4). On the opposite side, in which the medial forebrain bundle has been spared, there is no evidence of cellular change. In experiment PG 27 with a unilateral lesion in the septum that involves both the medial and the lateral septal nuclei (Text-fig. 3), there is a marked cell loss with shrinkage and pallor of the surviving cells in the nucleus of the diagonal band. As the medial forebrain bundle is traced into the lateral hypothalamus the cell changes become markedly reduced but are heavier again in the supramamillary region, particularly its lateral part, and in the ventral tegmental area.

The lesion in PG 6 is valuable in that it gives the rostral limit of the projection from the ventral tegmental area. In this brain the centrum ovale, the nucleus accumbens and the olfactory tubercle are destroyed back to the level of junction of the rostral and middle thirds of the septum (Text-fig. 3). There is no evidence of cellular degeneration in either the supramamillary region or the ventral tegmental area in this brain.

The rabbit material adds little to these observations as the majority of the lesions in these experiments lie either in, or close to, the ventral tegmental area. However, in two experiments, R 15 and R 17, the lesion extends no further posteriorly than the supramamillary region and in both there is definite cell shrinkage in the ventral tegmental area, which suggests that the rostral projection of the ventral tegmental area in the rabbit closely resembles that found in the rat.

The tegmental reticular nucleus

In a number of the rat brains degeneration has been seen in the tegmental reticular nucleus. Since all the lesions lie rostral to this nucleus the following account will be concerned only with the rostral projection; for an account of its other connexions reference should be made to Brodal (1957).

In experiment PG 28 there is extensive cell shrinkage in the anterior two thirds of the right tegmental reticular nucleus (Pl. 3, fig. 6). The lesion damages the left periaqueductal grey medial to the habenulo-peduncular tract and ventral to the posterior commissure. It also lies in the supramamillary region close to the midline, but involves rather more of this region on the right side than on the left. Further rostrally the lesion is confined to the right and includes the region of the principal mamillary tract. In this position it damages many of the periventricular fibres that pass from the posterior hypothalamus, but spares the medial forebrain bundle as it passes through the supramamillary region. The lesion ends in the zona incerta and ventral thalamic nucleus of the right side close to the mamillo-thalamic tract (Text-fig. 2).

There is a similar degree of shrinkage in the rostral three-quarters of the left tegmental reticular nucleus in experiment PG 39. In this brain the most caudal part of the lesion is a small incision through the medial mamillary nucleus and the medial supramamillary region of the left side. Further rostrally the periventricular fibres of the left posterior hypothalamus are damaged before the lesion crosses the midline to end as an extensive area of destruction between the dorsomedial thalamic nucleus and the ventromedial hypothalamic nucleus of the right side.

While these two experiments suggest that the relevant fibres from the tegmental reticular nucleus pass through the medial supramamillary region or the adjacent periventricular region of the posteromedial hypothalamus, it should be pointed out that the results in other experiments are more equivocal, and at present the details of the connexions between the tegmental reticular nucleus and the hypothalamus must remain in some doubt.

The nuclei of the raphe

Although a study of the rostral projection of the raphe nuclei is beyond the scope of this investigation it may be noted that in several experiments degenerative changes, in the form of some cell loss and some cell shrinkage, have been found in the nuclei of the raphe, particularly in the dorsal nucleus of the raphe (Pl. 3, fig. 5) and nucleus centralis superior. Our experiments do not permit an analysis of either the course or the termination of the fibres from these nuclei, but they do show that some of these nuclei are projecting rostrally into the diencephalon, thus confirming the observations of Brodal, Taber & Walberg (1960).

DISCUSSION

In view of the marked variation in the cellular degeneration found in the different nuclear groups it is necessary to consider their contribution to the three ascending pathways to the hypothalamus separately.

The deep tegmental nucleus

The finding of cellular degeneration in the deep tegmental nucleus after lesions of the mamillary peduncle of the rat and rabbit is in agreement with the observations of Quensel (1911) in the rabbit, and of Fox (1941) and Akert & Andy (1955) in the cat. The complete cell loss in this nucleus after section of the peduncle indicates that it is projecting principally into this bundle, and it is clear that it forms a major source of afferents to the mamillary nuclei. In the rat the fibres from the deep tegmental nucleus appear to pass in the medial part of the peduncle, and to end predominantly in the medial mamillary nucleus. Thus lesions confined to the medial mamillary nucleus produce cellular degeneration in the deep tegmental nucleus, although such limited lesions have never produced the complete cell loss in the nucleus that is seen after section of the mamillary peduncle, a number of pale-staining shrunken neurons always survive. It therefore seems possible that the deep tegmental nucleus is also contributing fibres, probably collaterals, to the lateral mamillary nucleus or medial forebrain bundle.

The dorsal tegmental nucleus

The degenerative changes found in the dorsal tegmental nucleus after mamillary peduncle lesions are in agreement with the earlier observations of Akert & Andy (1955). Although Fox (1941) made no mention of these changes in his account of the origin of the mamillary peduncle in the cat, his figure 3 suggests that the dorsal tegmental nucleus may well have been affected in his experiments. The appearance of the cellular degeneration in this nucleus is in marked contrast to that found in the deep tegmental nucleus. In both the rabbit and the rat the degeneration in the pars centralis is seen as cell shrinkage with no appreciable cell loss, regardless of the length of the post-operative survival or the severity of the lesion. Two completely distinct interpretations may be put forward to account for such degenerative changes. The first, and most probable, explanation is that the surviving cells give of collaterals to other sites, and that these are responsible for maintaining the viability of the cells after lesions of the mamillary peduncle. This explanation has been advanced to account for the absence of degenerative changes in neocortical and hippocampal pyramidal cells (Cajal, 1909; Daitz & Powell, 1954), and for the persistence of shrunken neurons in the thalamus and brainstem reticular formation (Walker, 1938; Bodian, 1940; Powell, 1952; Brodal, 1957). That the cells of the dorsal tegmental nucleus have such collaterals has previously been suggested by Morest (1961); after lesions of this nucleus he has described fibre degeneration not only in the mamillary and more rostrally situated nuclei, but also in the deep tegmental nucleus, the nucleus centralis superior, the tegmental reticular nucleus and the periaqueductal grey. Although the existence of collaterals would provide an explanation for the cell changes found in the dorsal tegmental nucleus it must be stressed that not sufficient is known at present about the nature of retrograde cell degeneration for it to be possible to assume that collaterals are invariably responsible for cell survival. Collaterals provide the simplest explanation of such survival, but their independent demonstration is of importance not only to clarify the connexions of the dorsal tegmental nucleus, but in general to elucidate the nature of the retrograde degenerative

process. An alternative explanation which has to be considered when the predominant change is that of cell shrinkage rather than cell loss is that the degeneration is transneuronal in character (Cook, Walker & Barr, 1951; Matthews, Cowan & Powell, 1960; Powell & Erulkar, 1962). In the dorsal tegmental nucleus, however, this possibility can probably be discounted even though there is evidence that this nucleus receives mamillotegmental fibres. First, the pattern of degeneration in the nucleus does not conform to what is known of the organization of the mamillotegmental projection (both the pars centralis and the ventromedialis show changes in the present experiments, but only the former receives afferents from the mamillary nuclei—Guillery, 1957), and secondly, in rabbit R 56 the pars centralis shows the characteristic cell shrinkage after a lesion which has spared the mamillotegmental tract.

Lesions confined to the medial mamillary nucleus have not produced any cellular change in the dorsal tegmental nucleus, although Guillery (1956) and Morest (1961) have shown that lesions in the dorsal tegmental nucleus produce fibre degeneration in the medial mamillary nucleus. Cajal (1911) showed that the individual fibres of the mamillary peduncle branch just dorsal to the lateral mamillary nucleus, and Morest found that the pars centralis of the dorsal tegmental nucleus projects to the medial forebrain bundle in addition to its medial mamillary ending. It thus seems probable that the contribution to the medial forebrain bundle was sufficient to maintain the cells of the pars centralis in the experiments in which the medial mamillary nucleus only was damaged.

Morest found that the pars ventromedialis projects to the lateral mamillary nucleus, but found no projection to the medial forebrain bundle from this part. The present results show that the pars ventromedialis sends some fibres or collaterals into the medial forebrain bundle, and at present this discrepancy cannot be resolved.

The ventral tegmental area

Fox (1941) has described cell loss in the ventral tegmental area after a lesion that included the mamillary nuclei and the lateral part of the posterior hypothalamus, and has discussed the possibility that this area contributes fibres to the mamillary peduncle. As the changes in the ventral tegmental area are not significantly greater after lesions at the level of the mamillary bodies than after lesions of the premamillary hypothalamus it must be concluded that the majority of the fibres from this area pass rostrally into the medial forebrain bundle, and that at present there is no evidence that any of these fibres enter the mamillary bodies. As the ventral tegmental area receives descending connexions from the medial forebrain bundle and fornix (Guillery, 1957; Nauta, 1958) the possibility that the changes that have been seen in this area are transneuronal, again cannot be completely excluded.

Until more is known of the relationship between the mamillary peduncle and the medial forebrain bundle it is convenient to distinguish between those mesencephalic nuclei which contribute fibres, either directly or by way of collaterals, to the mamillary nuclei, and those which appear to project exclusively to the premamillary hypothalamus. The former can be regarded as contributing to the mamillary peduncle, and the latter can be regarded as a part of the medial forebrain bundle. On these grounds the ventral tegmental area, though topographically related to the mamillary

peduncle, should be considered as an integral part of the medial forebrain bundle. This bundle forms a system of ascending and descending fibres that lie interspersed among the cells from which they take origin. Studies with the Nauta method show that the length of the fibres is highly variable, some passing for only a short distance in the bundle, while others may extend all the way between the septum rostrally and the midbrain caudally (Guillery, 1957; Nauta, 1958; Nauta & Kuypers, 1958). The pattern of cellular degeneration found in the lateral hypothalamus, lateral supramamillary region and ventral tegmental area suggests that the majority of the long ascending fibres come from the most caudal area, but that cells throughout the whole length of the system contribute some ascending fibres. Further, as the predominant cell change has always been shrinkage rather than cell loss it is possible that many of the cells in these areas have axons which divide into ascending and descending branches. A study of the medial forebrain bundle by Golgi methods would prove of considerable interest from this point of view.

Other ascending connexions

The degeneration seen in the tegmental reticular nucleus in our rat material suggests that this nucleus has connexions with the hypothalamus, but the fact that this nucleus is known to contribute a number of fibres to the cerebellum (Brodal, 1957) and also that no degeneration is seen in this nucleus after hypothalamic lesions in the rabbit raises several problems. There may be a species difference in the connexions or cellular reactions of this nucleus, and it should be noted that Brodal & Rossi (1955) did not describe degeneration in this nucleus after a hemisection of the brainstem at the di-mesencephalic junction in the cat, and also that, according to Brodal, the entire nucleus degenerates after cerebellectomy in the cat.

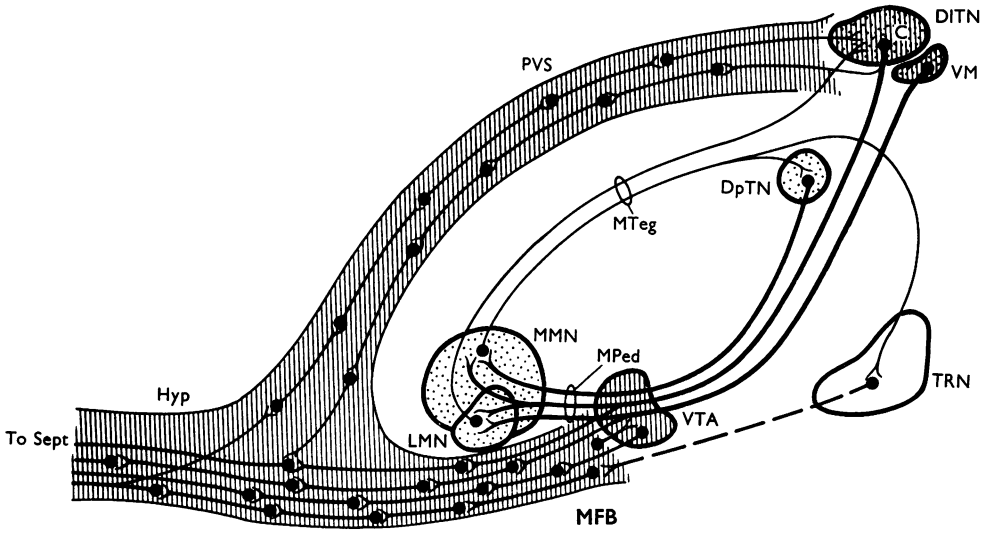
The absence of cellular degeneration in other nuclei of the mesencephalic and pontine tegmentum does not by itself exclude the possibility that they send axons, or collaterals, to more rostral levels. Thus, our failure to find clear-cut changes in such nuclei, as described by Brodal (1957) in the cat, does not exclude a rostral projection from these areas, and it is significant that in our experiments most of the animals used were adults and were allowed to survive for long periods, whereas Brodal used his more sensitive modification of the Gudden technique.

General conclusions

In Text-figure 6 an attempt has been made to summarize the present findings in conjunction with earlier fibre degeneration studies, with respect to the interrelationships between the major pathways linking the midbrain to the hypothalamus. From this it may be seen that the deep tegmental nucleus appears to project exclusively to the mamillary nuclei through the mamillary peduncle while the ventral tegmental area (and the tegmental reticular nucleus) appear to have no connexion with the mamillary nuclei but are an important source of afferents to the premamillary hypothalamus through the medial forebrain bundle. The central and ventromedial parts of the dorsal tegmental nucleus, on the other hand, have a dual relationship in that they project both to the mamillary nuclei and to more rostral levels through the medial forebrain bundle and the periventricular fibre system; it is of interest that these nuclei also appear to differ in their afferent connexions. The deep tegmental

nucleus is known only to receive afferents from the mamillary nuclei, while the ventral tegmental area receives most of its descending afferents by way of the fornix and the medial forebrain bundle; the dorsal tegmental nucleus has been shown to receive descending connexions by way of the periventricular system as well as from the mamillary nuclei by way of the mamillotegmental tract. Thus, the deep tegmental nucleus has mainly mamillary connexions, the ventral tegmental area has mainly premamillary connexions and the dorsal tegmental nucleus appears to be largely a region of interchange between the mamillary and premamillary connexions.

The reciprocal connexions that are formed between the mamillary nuclei and the tegmental nuclei of Gudden show that these cell groups are capable of a variety of



Text-fig. 6. Diagram to show the main ascending fibre pathways that link the hypothalamus and the midbrain. Areas that are primarily linked with the medial forebrain bundle and the periventricular fibre system are shown by cross-hatching, while areas that are primarily connected with the mamillary bodies are shown by stippling.

ordered interactions. Whatever the pattern of this interaction may prove to be, and even the known possibilities have been simplified in the diagram, its relevance to the rest of the nervous system can only be understood in terms of the connexions that do not return to the mamillary or tegmental nuclei. Text-figure 6 shows that such connexions are formed in the dorsal tegmental nucleus and in the ventral tegmental area, where the mamillary system is linked to the periventricular system and to the medial forebrain bundle. The figure shows the extensive interchange that occurs between the medial forebrain bundle and the periventricular fibres at posterior hypothalamic levels, and indicates that both systems have the same basic structure of cells mingled with ascending and descending fibres, the length of the fibres being highly variable. From the point of view of these fibre connexions the mamillary bodies can be seen as an integral, if somewhat specialized part of the hypothalamus. It is necessary to stress this because recent discussion has tended to obscure this essential relationship, by emphasizing the discreteness of the mamillary nuclei as a

group that is connected to the rest of the brain by morphologically well-defined fibre tracts. The present observations have shown that at least the mesencephalic connexions of the mamillary bodies overlap, and hence are presumably able to interact, with a more general ascending and descending system, which includes the medial forebrain bundle and periventricular system, and which links the premamillary hypothalamus to the midbrain.

SUMMARY

The method of retrograde cell degeneration has been used to study the mesencephalic origin of fibres to the hypothalamus in the rat and rabbit.

Ascending fibres have been found to arise in the deep and dorsal tegmental nuclei, the ventral tegmental area, and the tegmental reticular nucleus.

The deep tegmental nucleus projects through the mamillary peduncle, mainly to the rostral part of the medial mamillary nucleus, and shows complete cell loss after interruption of the mamillary peduncle. The dorsal tegmental nucleus appears to be connected with both mamillary nuclei, but the form of the cellular degeneration (shrinkage and pallor of the cells rather than cell loss) in this tegmental nucleus suggests that collaterals are given off to the premamillary hypothalamus through the medial forebrain bundle and to other mesencephalic nuclei.

The ventral tegmental area and the tegmental reticular nucleus project to the premamillary hypothalamus through the medial forebrain bundle and the periventricular system.

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REFERENCES

AKERT, K. & ANDY, O. J. (1955). Experimental studies on corpus mamillare and tegmento-mamillary system in the cat. *Amer. J. Physiol.* **183**, 591.

BODIAN, D. (1940). Studies on the diencephalon of the *Virginia oppossum*. Part II. The fibre connections in normal and experimental material. *J. comp. Neurol.* **72**, 207–297.

BRODAL, A. (1957). *The reticular formation of the brain stem. Anatomical aspects and functional correlations.* Edinburgh: Oliver and Boyd.

BRODAL, A., POMPEIANO, O. & WALBERG, F. (1962). *The vestibular nuclei and their connexions. Anatomy and functional correlations.* Edinburgh: Oliver and Boyd.

BRODAL, A. & ROSSI, G. F. (1955). Ascending fibres in brain stem reticular formation of cat. *Arch. Neurol. Psychiat., Chicago*, **74**, 68–87.

BRODAL, A., TABER, E. & WALBERG, F. (1960). The raphe nuclei of the brain stem in the cat. II. Efferent connections. *J. comp. Neurol.* **114**, 239–259.

CAJAL, S. R. (1909). *Histologie du système nerveux de l'homme et des vertébrés. Vol.* 1. Paris: A. Maloine.

CAJAL, S. R. (1911). *Histologie du système nerveux de l'homme et des vertébrés. Vol.* 2. Paris: A. Maloine.

COOK, W. H., WALKER, J. H. & BARR, M. L. (1951). A cytological study of transneuronal atrophy in the cat and rabbit. *J. comp. Neurol.* **74**, 68–87.

COWAN, W. M. & POWELL, T. P. S. (1955). An experimental study of the relation between the medial mamillary nucleus and the cingulate cortex. *Proc. Roy. Soc.* B, **143**, 114–125.

DAITZ, H. & POWELL, T. P. S. (1954). Studies on the connexions of the fornix system. *J. Neurol.* **17**, 75–82.

FOX, C. A. (1941). The mamillary peduncle and ventral tegmental nucleus in the cat. *J. comp. Neurol.* **75**, 411–425.

GILLILAN, L. A. (1943). The nuclear pattern of the non-tectal portions of the midbrain and isthmus in rodents. *J. comp. Neurol.* **78**, 213–251.

GUILLERY, R. W. (1956). Degeneration in the post-commissural fornix and the mamillary peduncle of the rat. *J. Anat., Lond.*, **90**, 350–370.

GUILLERY, R. W. (1957). Degeneration in the hypothalamic connexions of the albino rat. *J. Anat., Lond.*, **91**, 91–115.

GURDJIAN, E. S. (1927). The diencephalon of the albino rat. *J. comp. Neurol.* **43**, 1–114.

INGLE, D. J. & GRIFFITH, J. (1942). The surgery of the rat. In: *The Rat in Laboratory Investigation.* Ed. J. Q. Griffith and E. J. Farris. Philadelphia: Lippincott.

MATTHEWS, M. R., COWAN, W. M. & POWELL, T. P. S. (1960). Transneuronal cell degeneration in the lateral geniculate nucleus of the macaque monkey. *J. Anat., Lond.*, **94**, 145–169.

MEESEN, H. & OLSZEWSKI, J. (1949). *A Cytoarchitectonic Atlas of the Rhombencephalon of the Rabbit.* Basel–New York: S. Karger.

MOREST, D. K. (1961). Connexions of the dorsal tegmental nucleus in the rat and rabbit. *J. Anat., Lond.*, **95**, 229–246.

NAUTA, W. J. H. (1958). Hippocampal projections and related neural pathways to the midbrain in the cat. *Brain*, **81**, 319–340.

NAUTA, W. J. H. & KUYPERS, G. J. M. (1958). Some ascending pathways in the brain stem reticular formation. In *Reticular Formation of the Brain.* Boston: Little, Brown and Co.

PAPEZ, J. W. (1932). The nucleus of the mamillary peduncle. *Anat. Rec.* **52**, 72–73.

POWELL, T. P. S. (1952). Residual neurons in the human thalamus following hemidecortication. *Brain*, **75**, 571–584.

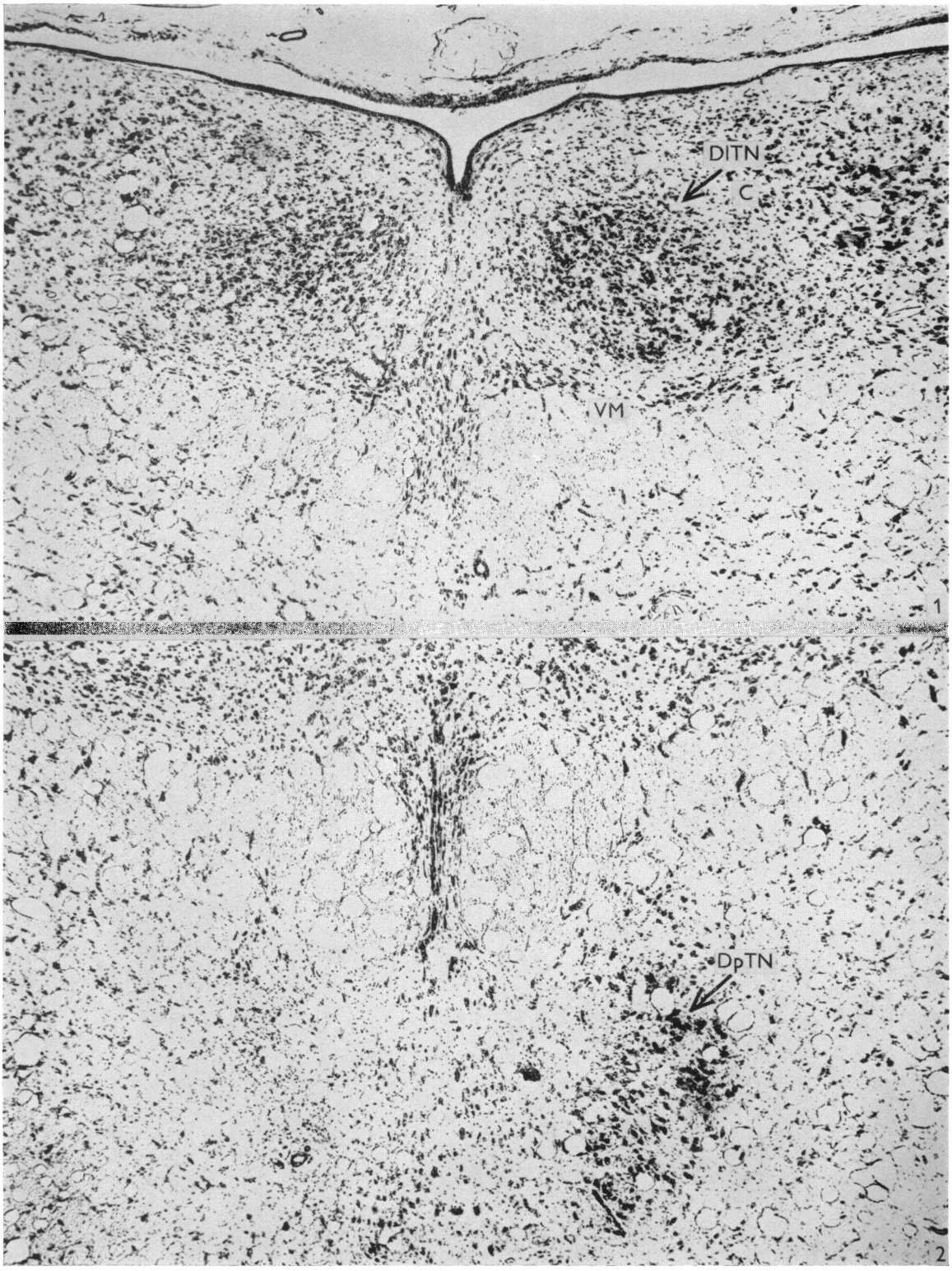
POWELL, T. P. S. & ERULKAR, S. D. (1962). Transneuronal cell degeneration in the auditory relay nuclei of the cat. *J. Anat., Lond.*, **96**, 249–268.

QUENSEL, F. (1911). Untersuchungen uber die Tektonik von Mittle- und Zwischenhirn des Kaninchens. *Pflüg. Arch. ges. Physiol.* **139**, 47–92.

WALKER, A. E. (1938). *The Primate Thalamus.* University of Chicago Press.

LIST OF ABBREVIATIONS

AC	Anterior commissure
AHA	Anterior hypothalamic area
AON	Anterior olfactory nucleus
C	Pars centralis of the dorsal tegmental nucleus
CC	Corpus callosum
CO	Centrum ovale
CP	Cerebral peduncle
CS	Corpus striatum
DlTN	Dorsal tegmental nucleus
DM	Dorsomedial hypothalamic nucleus
DNR	Dorsal nucleus of raphe
DpTN	Deep tegmental nucleus
DT	Dorsal thalamus
F	Fornix
GP	Globus pallidus
Hip	Hippocampus
HPT	Habenulo-peduncular tract
Hyp	Hypothalamus
IC	Inferior colliculus
IPN	Interpeduncular nucleus
LHA	Lateral hypothalamic area
LMN	Lateral mamillary nucleus
LPA	Lateral preoptic area
LSN	Lateral septal nucleus
MCP	Middle cerebellar peduncle
MFB	Medial forebrain bundle
MGB	Medial geniculate body
MLB	Medial longitudinal bundle
MMNb	Pars basalis of the rabbit medial mamillary nucleus
MMNl	Medial mamillary nucleus pars lateralis
MMNm	Medial mamillary nucleus pars medialis
MMNp	Medial mamillary nucleus pars posterior
MPed	Mamillary peduncle
MPA	Medial preoptic area
MSN	Medial septal nucleus
MTeg	Mamillotegmental tract
MTT	Mamillothalamic tract
NA	Nucleus accumbens
NDB	Nucleus of diagonal band
NMPl	Nucleus mesencephalicus profundus lateralis
OC	Optic chiasma
OT	Optic tract
P	Pyramidal tract fibres
PAG	Periaqueductal grey
PC	Pyriform cortex
PCm	Posterior commissure
PN	Pontine nuclei
PO	Posterior complex of thalamus
PVN	Paraventricular nucleus
PVS	Periventricular system
RN	Red nucleus
SC	Superior colliculus
SCN	Suprachiasmatic nucleus
Sept	Septum
SFN	Septofimbral nucleus
SN	Substantia nigra
SON	Supraoptic nucleus
TO	Olfactory tubercle
TRN	Tegmental reticular nucleus
VM	Pars ventromedialis of the dorsal tegmental nucleus
VTA	Ventral tegmental area
III	Oculomotor nerve or nucleus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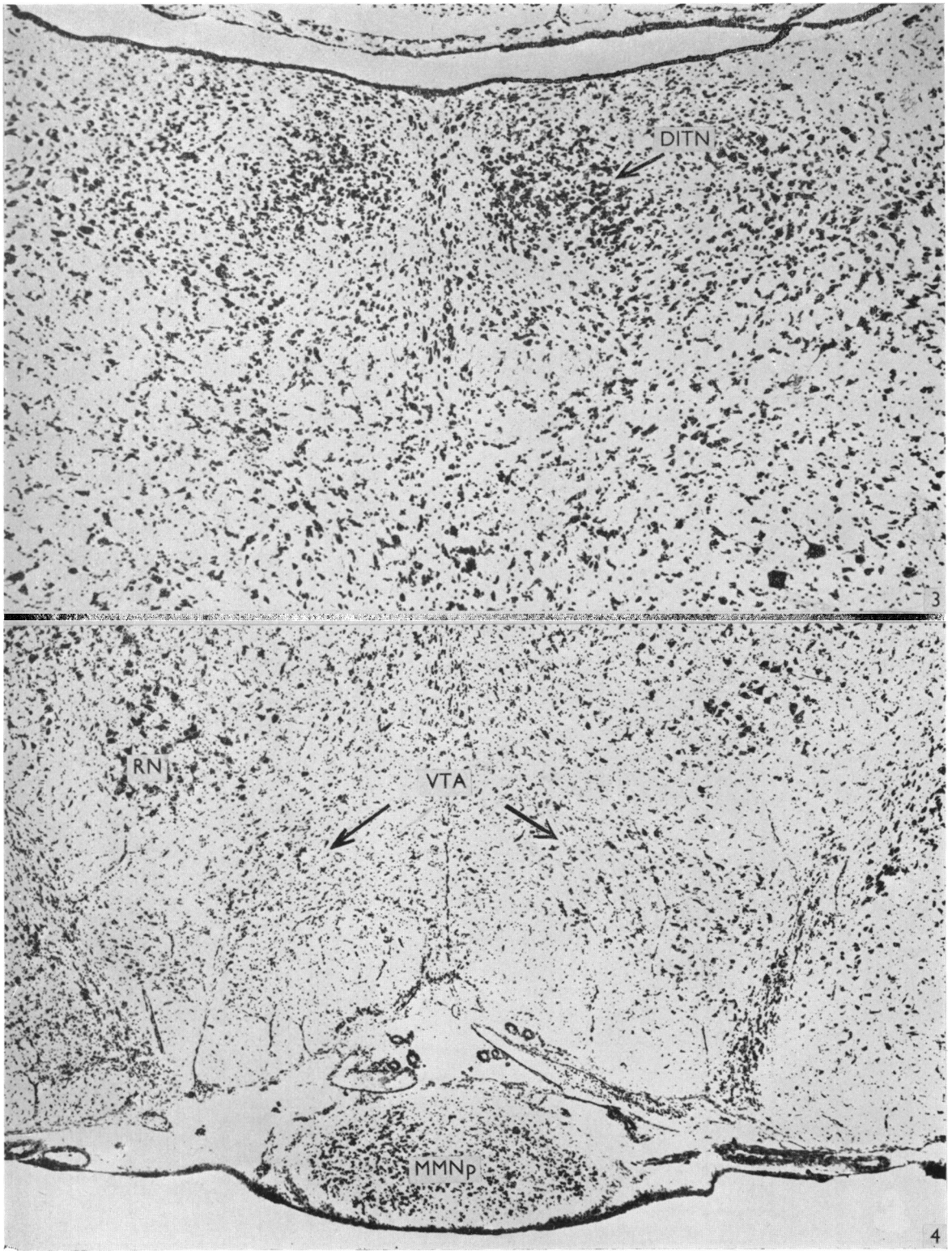
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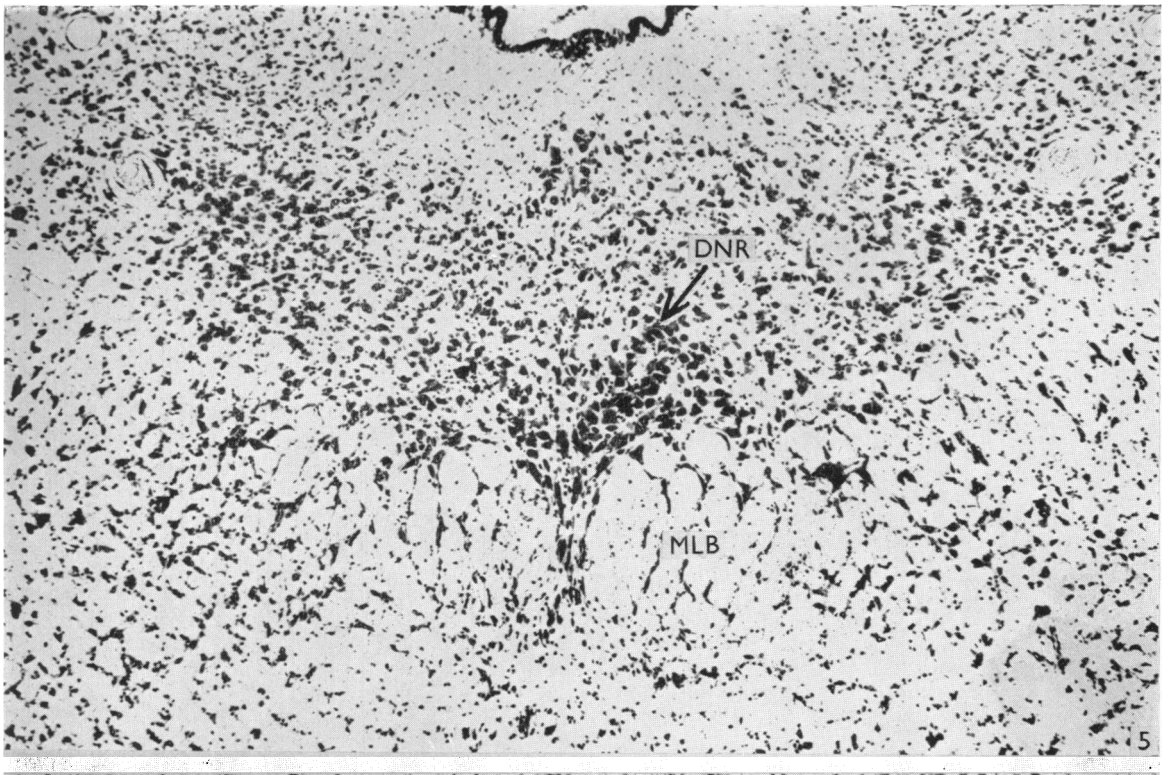
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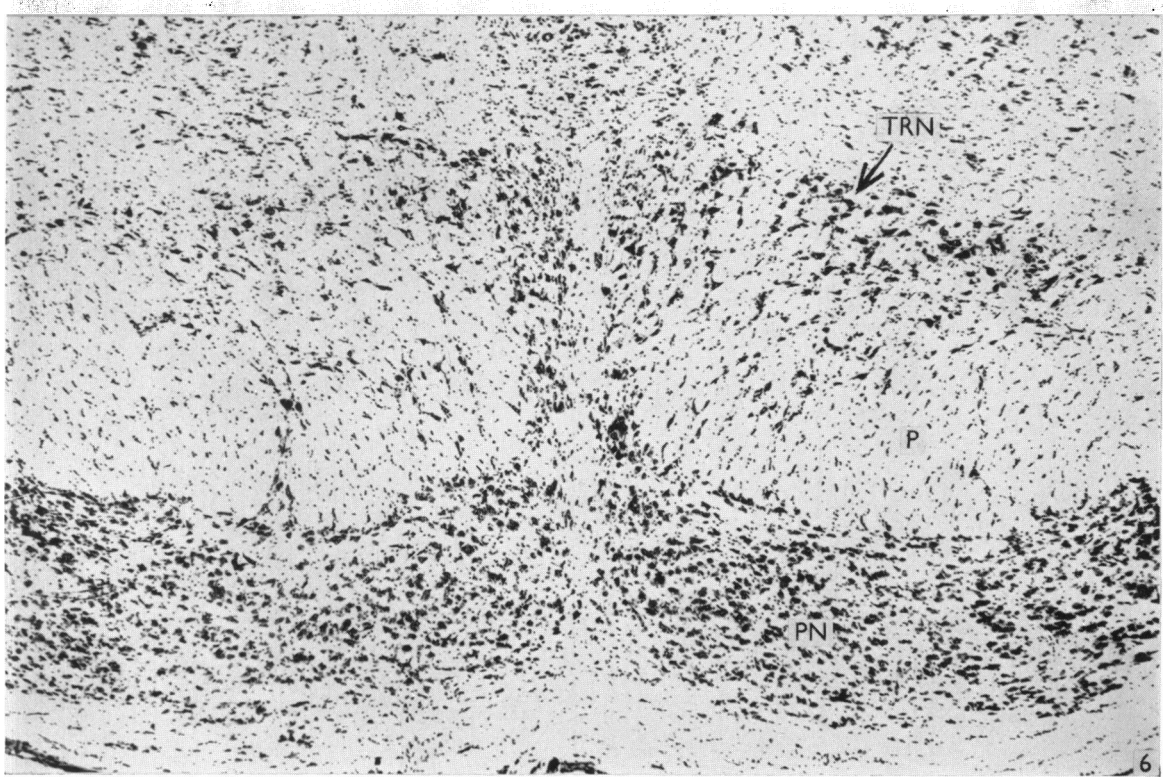
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EXPLANATION OF PLATES

All photomicrographs are of thionin-stained sections.

PLATE 1

Fig. 1. A low-power photomicrograph to show the degree of cell shrinkage in the dorsal tegmental nucleus of the right side in rabbit R17 2 months after interruption of the mamillary peduncle. The arrow points to the normal nucleus of the left side. × 66.

Fig. 2. A comparable view of the deep tegmental nucleus in this experiment. Apart from a few shrunken cells in its dorsolateral part the nucleus on the right side appears to have undergone complete cell loss. × 66.

PLATE 2

Fig. 3. To show the degree of cell shrinkage in the dorsal tegmental nucleus on the right side in the rat 1 month after a lesion of the mamillary peduncle (experiment PG28). × 22.

Fig. 4. A low-power view of the ventral tegmental area in experiment PG32 (1 month survival). Observe the marked cell shrinkage in this area on the right side as compared with the normal area on the left. Arrows point to the ventral tegmental area on both sides. × 82.

PLATE 3

Fig. 5. To show the cellular degeneration which has occurred in the dorsal nucleus of the raphe of the right side in experiment PG29. × 90.

Fig. 6. A low-power view of the tegmental reticular nuclei in experiment PG28. Observe the marked cell shrinkage which has occurred in the nucleus of the right side. × 64.