HS

THE MORPHOLOGY AND CONNECTIONS of the HABENULA

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CHAPTER I

INTRODUCTION

Literature Review

The habenular nuclei are two small gray masses forming triangular eminences on the dorsomedial surface of the thalami. They are marked dorsally as slightly elevated, rounded arms embracing the posterior superior part of the third ventricle. As the arms continue rostrally, they become thin and merge on either side into a longitudinal ridge formed by the striae medullares thalami.

From the time of Cajal's description ('11) through that of Kappers, Huber and Crosby, it has been customary to regard the habenula as composed of two cell groups (A Med. and Lat.). Kappers et al described this nucleus in almost the same way as did Cajal, except that the former investigators found the cells of the lateral group to be smaller than those of the medial. Gurdjian (rat), Tsai (opossum) and Papez (armadillo) subdivide the medial nucleus into a ventromedial and a dorsomedial part. Gurdjian describes the ventromedial portion in close relation to the ventricular cavity, where it is located in the rostral third of the habenular complex. Its cells are compactly placed in the form of an irregular triangular mass. This becomes larger caudally and eventually joins the dorsomedial portion of

the medial nucleus to form the medial nucleus of classical description. The dorsomedial portion of the medial habenula consists of a group of cells loosely placed, the group being dorsal and somewhat lateral to the ventromedial portion.

In the lateral nucleus, Papez ('32) distinguishes two extreme varieties of cells: a moderate number of large cells somewhat globular in shape and a large number of small round cells. Cells of intermediate size are also numerous. Silver sections show a dense pericellular network in the lateral habenular nucleus intimately united with the adjoining part of the middle portion of the medullary stria, which sends many fiber bundles as well as collaterals into it. Many fine fibers, probably from the small cells, appear to unite the lateral nucleus with the lateral part of the medial habenular nucleus. More recently, Bodian ('39) using various stains describes both the medial and lateral nucleus of the habenula of the opossum as subdivided into two parts. The lateral nucleus is composed of a dorsal portion, containing large cells, and a ventral portion composed of smaller and more scattered cells.

Ingram ('32), in the cat, describes the cells of the medial habenular nucleus as closely packed, of medium size, rounded to triangular, with deeply stained cytoplasm and oval nuclei. The lateral extends farther rostrally than the medial and its cells stain much less deeply than those of the latter. The cells are of two sizes, small and medium; their shape is rounded to triangular and they contain large, pale, oval nuclei.

Walker ('38), in examining the thalamus in primates, did not mention any particular cell groups.

Marburg ('44) with human material in Nissl and Weigert-Pal preparations, found the habenula to consist of five cellular groups. These are: I. a ventromedial small-celled, and II. a medial (central) small-celled group, III. a dorsomedial small-celled group, IV. a dorsolateral group of small and medium-sized cells and V. a lateral large-celled group.

The ventromedial small-celled group is the first to be seen rostrally and can be followed as far back as the region of the posterior commissure. It tapers off at both its rostral and caudal limits. This group has a very characteristic arrangement of closely packed, very small cells in which neither the nuclei nor the protoplasm are clearly visible. The cells are spindle-shaped and clearly separated from the paramedian cell group.

Dorsolateral to this group lies the medial (central) group (II). It does not extend as rostrally as the ventromedial small-celled group but begins as a few scattered cells which rapidly increase in number toward the middle of the nucleus and which can be followed into the region of the posterior commissure. The cells of this nucleus are somewhat larger and fewer than those of the ventromedial. They are also spindle-shaped but have distinct nuclei and better developed protoplasm. Some large cells are found in the lateral and dorsal parts of the central group where

this part comes in contact with the larger cells of the lateral group.

Dorsal to the two described groups lies a third small-celled nucleus, the dorsomediam (III). In size and shape its cells resemble those of group II and are seen at the same levels as the two groups mentioned earlier.

The lateral part of the habenula is composed of two parts, the more medial of which, the dorsolateral group (IV), forms a wedge between the magnocellular lateral nucleus (V) and groups II and III. The base of this wedge (IV) is directed dorsally. The borderlines between it and the medial group (III) are indistinct. This group has the shortest anteroposterior extent. Most of its cells are of medium size, polygonal, contain well-developed nuclei and show prominent tigroid bodies. The numerous small cells within this group resemble cells of group II.

The second lateral group (V) is represented by the largest cells of the habenula. In certain locations it consists of a dorsal and a ventral part; the former elevates the dorsal surface of the habenula. This group is less well developed rostrally than any of the other groups, but is the last to disappear in the region of the posterior commissure.

The fine structure of the habenula, as revealed in Golgi preparations has been described by Cajal. He describes the cells of the medial nucleus as small with stellate shapes. Their dendrites, two, three or sometimes more, are short. They

course in various directions, and each of them terminates by means of a considerable number of small branches which have a coarse outline, are sometimes thorny, and follow a very irregular course. The axon of these cells is very thin and leaves the cell body, although sometimes it can also originate in a dendrite. In the adult cells, its course is very complicated; it goes up and down, outward and inward. Eventually it becomes vertical and enters Meynert's fasciculus at the level of the ventrolateral pole of the nucleus. "In the cat and dog, these fibers are provided with collaterals (one, two or three) which branch and seem to be confined to the region of the medial nucleus itself, possibly establishing contacts with the pools of cells from which they come."[1]

The medial nucleus of the habenula is filled with the nerve endings of the stria medullaris. These are thick fibers which do not lose their myelin until they approach their terminal arborization. Instead of the wide diffuse branching which is characteristic of the termination of the fibers in the cerebrum and in most central gray nuclei, there is a very thick arborization which can be compared to the fibers that surround the cells of Purkinje in the cerebellum. "In certain places this branching is so rich that it looks like a bush where the course of the secondary fibers cannot be followed. As a rule, before giving off the terminal arborization, the fiber becomes thicker."[2] It forms two or three preterminal branches that, in turn, become immediately intermingled filaments. Therefore, each individual

fiber ends in several nerve cells. The pattern of these endings is similar in all the mammals (mouse, rabbit, dog, and cat), that Cajal studied.

The cells of the lateral nucleus are star shaped and have long processes that make them look like most of the cells found in the other nuclei of the thalamus. These cells are intermingled with bundles of fibers which run in the superior and external portion in an antero-posterior direction. Such bundles correspond to the lateral half of the stria medullaris.

The axons of these cells are thick and give off three, four, or more collaterals which are distributed within the nucleus itself.

The afferent endings are thin fibers which branch in a diffuse manner. Many of the branches represent the termination of the fibers that make the external component of the stria medullaris. This loose type of arborization is so different from the one found in the medial nucleus that it is possible that the nervous impulse carried by each fiber may not confine its influence to a small number of cells, as the medial, but that it may also affect nearly all the nerve cells of the nucleus.

The larger neurons are about 15 by 20 u in diameter and the smaller ones about 10 u in diameter. Electron microscopic examination shows that there is no significant difference between the two sizes of neurons except the amount of cytoplasm (Milhaud, 1966). The usual cytoplasmic organelles, such as golgi apparatus, mitochondria, granular endoplasmic reticulum

and occasional dense core bodies are present. Dendritic trunks, some of which are very large, are characterized by their high content of microtubules and long mitochondria. Milhaud reports seeing dendritic spines with some frequency in the habenula but does not make a distinction whether they are found medially, laterally, or both. They are always found in sections with two axons surrounding the neck and forming synapses on both sides but not at the bulbous tip. Axons forming synaptic junctions mostly occur on dendritic trunks regardless of their size; fewer are seen on neuronal somata and on dendritic spines; rarely are any seen on other axons.

It would be interesting to know what is the functional significance of these two nuclear groups in the habenula.

Moliner ('62) has proposed a system of classification of nerve cells on the basis of their dendritic patterns which suggest the existence of a certain correlation between morphology and function. In his study he used the brains of mice, rats, cats and monkeys stained with modifications of the Golgi and Golgi-Cox methods which give a consistent impregnation of the dendrites of most parts of the central nervous system.

The branching architecture of dendrites has permitted their classification into generalized and specialized patterns: (1) the generalized or radiate pattern. This might be considered as the prototype from which all other dendritic patterns can be derived. It is a generalized pattern with relatively straight dendrites radiating in all directions. The dendrites of third

order are longer than those of second order and in turn, these are longer than those of first order. As a result, the dendritic field is looser in the periphery.

(2) The specialized pattern is generally tufted with few dendrites of first order which subdivide into many dendrites of secondary and higher orders.

The radiate type is widely distributed throughout the central nervous system. The neurons of the lateral nucleus of the habenula might be included in this pattern. The radiate pattern is also found wherever nerve cells and myelinated fibers of passage are freely intermingled. "Within the radiate type, a spectrum of subtypes can be distinguished on the basis of the size of the field covered by the dendrites, their number and the richness of arborization. The cells of the pars medialis and lateralis of the globus pallidus and of the hypothalamus typically show scarce and long dendrites."[3] Cells of such diverse locations are included under the radiate dendritic pattern because the latter may be related to an input of heterogeneous origin and/or the presence of relatively widely spaced afferent terminal fibers. The more specialized dendritic patterns seem to be present in regions frequently characterized by afferent connections of more homogeneous origin, composed of closely spaced axons that frequently terminate in dense clusters or other specialized endings. The tufted dendrites are characterized by the presence of a few dendrites of first order which subdivide into a multitude of secondary, tertiary and even quarternary branches so that each dendrite has the appearance of a broom or tuft. This tufted

appearance may result from either one of two different modes of ramification. Sometimes, there is an abrupt subdivision of a relatively small number of dendrites of first order into a large number of dendrites of second and third order. The tufted appearance is the result of this disproportion. In other cases, it is the result of a tree-type of branching; the daughter branches are shorter than the mother branch. This is the reversal of the formula that has been described for the radiate type. Moliner designates these two types as tufted I and tufted II. Tufted dendrites are present, though not exclusively, on the neurons of the sensory relay centers. Their number, size, polarity, and degree of straightness may vary, as well as the ratios between the various dendritic segments.

The neurons of the medial habenula can be included in this specialized dendritic pattern as described by Moliner.

Tufted dendrites of the type II are particularly evident in some secondary sensory nuclei, whereas those of type I are frequently found in the tertiary and quarternary sensory centers (e.g., thalamus).

Another type in this classification is the wavy type. This type is characterized by dendrites which follow a wavy course. Most of the cell groups which are believed to send fibers exclusively or preponderantly to the cerebellum contain neurons with dendrites displaying a varying degree of waviness; examples are the inferior olivary nuclei and lateral cuneate nuclei.

No clear-cut explanation has been offered for these observations but they appear to be related to the type of input or output associated with cell groups. "The tufted pattern seems to be characteristic of fairly well-defined regions with a relatively homogenous type of input, i.e. receiving axons from circumscribed cell groups often related to specific sense organs. For example, many of the generally recognized secondary sensory cell groups of the brain stem are composed mostly of neurons of this type, as are the so-called sensory relay nuclei of the thalamus."[4] In contrast to the tufted pattern, the radiate pattern is widely distributed. This is the only type present in the motor nuclei, as well as in other regions such as the brain stem reticular formation, which are known to have a multiplicity of afferent connections.

The main afferent bundle to the habenula is the stria medullaris thalami. The stria is a large and complex fasciculus which carries fibers from all parts of the olfactory field of the telencephalon to the habenula. According to Kappers, Huber and Crosby ('36) the component fiber tracts of the stria medullaris are: (1) the medial cortico-habenular tract, from the hippocampal region; (2) the septo-habenular tract, from the lateral septal nucleus; these fibers were derived from the large cells in the medial part of the septum and from the bed nucleus of the anterior commissure; (3) the amygdalo-habenular tract; (4) fibers from the stria terminalis, the bed nucleus of the stria and anterior commissure; (5) the lateral and medial olfacto-habenular

tracts, arising from periventricular, preoptic and hypothalamic regions and running with the lateral and medial forebrain bundles, respectively, before turning into the stria; (6) the lateral cortico-habenular tract, sometimes called lateral olfacto-habenular tract, from the piriform lobe and the nucleus of the lateral olfactory stria; (7) fibers from the tectum.

These fiber tracts appear to encompass all of the major portions of this fiber system which have been recognized throughout the vertebrate series.

Crosby ('17) and Huber and Crosby ('26) have described this stria in the alligator, and in birds ('29), and Hines ('29), in the Ornithorhynchus. Herrick ('13) made a preliminary analysis of the stria medullaris of the rat, and this has been amplified by Gurdjian ('25, '27), (from Loo, '31).

Primitively fibers converge into the stria medullaris in two main divisions, from the medial and lateral sides of the telencephalon; but in mammals these tracts become somewhat intermingled. The most primitive component is probably tractus olfacto-habenularis medialis passing directly dorsally from the central gray of the preoptic area to the habenula and ascending medially to the great basal forebrain bundles. Other similar fibers go up into the stria medullaris and pass laterally to these bundles -- tractus olfacto-habenularis lateralis. Fibers of this olfacto-habenular system arise also much farther forward from the region of the tuberculum olfactorium. On the medial side of the hemisphere a path from the bed nuclei in the lamina terminalis to the stria medullaris is probably also the primitive

tractus septo-habenularis (Loo, '31).

Although Kappers ('36) states that this tract originates from the lateral septal nucleus, Gurdjian thought these fibers were derived from the large cells in the medial part of the septum and from the bed nucleus of the anterior commissure. Nauta ('58) (cat) describes them as coming from the supracommissural part of the septal region to end in the medial nucleus of the habenula. Raisman ('66) confirms the termination of the septo-habenular fibers as described by Nauta ('56, '58) and Cragg ('61b), but limits the source to the septofimbrial nucleus, to the exclusion of the medial and lateral septal nuclei.

The union of the lateral cortico-habenular and the lateral olfacto-habenular tracts, on the one hand, with the septo-habenular, the stria terminalis connection, and the medial cortico-habenular tract, on the other hand, may be considered as marking the cephalad beginning of the stria medullaris. This union (Gurdjian) occurs in the rat near the anterior end of the diencephalon. The bundle thus formed is at first triangular in shape and passes along the extreme dorsomedial surface of the diencephalon toward the habenula. In its more caudal relations it is more circular in shape. In their course through the stria the various components maintain certain relationships to each other. Those from the dorsomedial wall of the hemisphere (the medial cortico-habenular and the septo-habenular) ocupy the dorsal half of the stria medullaris. The tracts from the lateral wall of the hemisphere and the ventral olfactory centers of the

medial part of the hemisphere and diencephalon (lateral cortico-habenular and the olfacto-habenular tracts) take a more ventral position, and are somewhat medially inclined. The medial olfacto-habenular tract has the most ventral position of any of the components. The stria medullaris fibers end for the most part in the habenular nuclei of the same side. A few may cross in the habenular commissure which is very small in the rat.

In the human, the stria medullaris consist of three different fiber groups with definite positions (Marburg, '44). "Dorsolaterally and laterally lie the amygdalo and pallido-habenular fibers; mediodorsally and ventrally, as well as centrally, the olfactory group is found, and ventrally the thalamic fibers and particularly those from the hypothalamus. Caudally the medial fibers of the olfactory group are supplanted by the commissural bundle which is formed by components from the ventral and central groups. Since there is no change in the position of the tracts of the stria at their level of entrance into the habenula, the medial and the dorsomedial nuclei probably represent end stations for the olfactory fibers, the dorsolateral nuclear group for the fibers from the stria cornea, the large-celled lateral nuclear group for the pallido-thalamic fibers, and the ventromedial small cells for the hypothalamic group. The other thalamic fibers probably also end in the medial (central) cell group, the ventral part of which is occasionally somewhat separated from the main nucleus."[5]

Ranson, in Marchi preparations, noted degeneration after

pallidal lesions over the width of the stratum zonale thalami, converging caudally into the stria medullaris and terminating in the lateral nucleus of the habenula. Nauta ('66) in Nauta-Gygax preparations of the monkey also notes evidence of pallidofugal fibers following the stratum zonale thalami to the habenula. The terminal arborizations appear to be limited to the lateral, magnocellular component of the lateral habenular nucleus. As previously mentioned, Marburg also found pallidohabenular fibers.

The dorsomedial nucleus of the thalamus receives afferent connections from sources similarly distributed to those of the habenular nuclei (Guillery, '59; Cragg, '61). Cragg, using rabbits, found that lesions in the preoptic area damaged fibers to the lateral habenular nucleus and also caused fiber degeneration in the dorsomedial nucleus. Frontal transections, however, interrupted a projection from frontal cortex to the dorsomedial nucleus but there was no counterpart in the lateral habenular nucleus. Septal lesions caused fiber degeneration in the dorsomedial nucleus but there was no concordance with involvement of the medial habenular projection (Cragg, '61).

The sources of fibers afferent to the habenular nuclei in the rabbit were not found to be as numerous as have been suggested by studies of normal material in cat and man (Cragg, '61). In the rabbit, the medial habenula nucleus apparently receives fibers from the posterior septum only and "fibers from the posterior septum do not reach the dorsal tegmental area

directly, but impulses would be relayed in the medial habenular or interpeduncular nuclei, or in both."[6]

Nauta ('56) reported that numberous fibers enter the stria medullaris thalami from the lateral preoptic region, and most of them terminate in the lateral habenular nucleus, but some fibers by-pass this nucleus to join the habenulo-peduncular tract and eventually enter the ventral tegmental area.

Since the area between the optic chiasma and the anterior commissure has been shown to respond to warming (Magoun, '38), it was suggested by Cragg that the lateral habenular nucleus may act as a relay nucleus for the temperature receptors in the preoptic area.

Some axons of the stria medullaris thalami pass over the habenular commissure. The habenular commissure is a system of transversal fibers situated in front and below the pineal body and seems to link the habenular nuclei of both sides as well as both stria medullares. According to Cajal this commissure does not receive any fibers either from the nuclei of the habenula or from the pineal body. It is only a partial decussation of the stria medullares. Before decussation, some of the fibers of the commissure send collaterals to the habenula of the same side. It is possible that all the fibers will behave in a similar manner and that the striae medullares of one hemisphere will supply both habenular nuclei on both sides. The course of these commissural fibers is not entirely transversal but arciform. They go backwards and inwards and then only inwards. They cross

the midline, forming a curve and its concavity facing forward and, finally, reach the most medial portion of the habenula of the opposite side where they terminate.

Marburg calls attention to a dorsal portion of thicker fibers and a ventral portion of finer fibers, and that some of these finer fibers descend into the medial part of the habenulo-peduncular tract.

Of additional interest, however, is a study of the secondary olfactory connections of Lacerta Viridis and Testudo Graeca (Gamble, '52a, '56). Gamble has shown by experimental degeneration that the olfacto-habenular tract runs in the opposite direction from the region of the habenular ganglion cranially, and that it consists of secondary olfactory fibers which have crossed in the habenular commissure. The habenular commissure, in this sense, may be considered an olfactory commissure.

So far as mammals are concerned, data is lacking concerning any direct connection from the olfactory bulb to the diagonal band or stria medullaris. "The only suggestion of any resemblance to the condition in Lacerta Viridis as regards a crossed system of secondary olfactory fibers, is found in Loo's ('31) account of the forebrain of the opossum. He described a component of the stria terminalis (stria terminalia 5) which joins the stria medullaris; its course and relations closely resemble those of the ventral division of Herrick's ('27) anterior olfacto-habenular tract."[7]

The chief efferent tract from the habenula is the

habenulo-peduncular tract or the fasciculus retroflexus of Meynert. This tract takes its origin from the lateral (or, according to Kappers, the lateral and medial) habenular nucleus and courses straight ventrally near the third ventricle, close to the red nucleus and finally reaches the interpeduncular nucleus between the cerebral peduncles, the fibers terminating in a characteristic corkscrew manner in all vertebrates (Kreig). This bundle also includes axons from the nucleus of the habenulo-peduncular tract located just ventral to the habenular groups, and some fibers from the pulvinar.

Efferents rostrally from the habenular nucleus, which terminate in the anterior hypothalamic area (Massopust, '62), have been described in the stria medullaris thalami (Mitchell, '61; Way, '67). Additional projections have been found to the preoptic region and the anterior amygdaloid area and eventually to the substantia innominata; a few fibers have also been observed to enter the pre- and post-commissural fornix and possibly the septal region (Mitchell, '63).

Nauta ('58) reported the results of a lesion restricted to the medial habenular nucleus and showed that there is massive degeneration in the compact part of the habenulo-peduncular tract. He suggests that the lateral part of this tract, which mainly by-passes the interpeduncular nucleus, originates in the lateral habenular nucleus.

Efferent fibers are also reported in the habenulo-peduncular tract from the interpeduncular nucleus and preterminal

degenerating fibers in the nucleus of this tract, in the medial dorsal and lateral dorsal nucleus and also in the habenula, more in its lateral nucleus (Mitchell, Massopust).

Akagi ('67) reports (after lesions in the medial habenular nucleus, which were stained with Nauta-Gygax technique) degenerating terminals in the parafascicular, hypothalamic, interpeduncular and ventral tegmental nuclei. There were terminals in the pretectum and lateral part of the superior colliculus.

After lesions in the lateral habenular nucleus, degenerating terminals were found in the parafascicular, central medial, hypothalamic, interpeduncular, dorsal raphe, ventral tegmental and dorsal tegmental nuclei.

Edinger ('11) was able to distinguish five parts in the interpeduncular nucleus. Cajal ('11) recognized in this nucleus an unpaired mass of gray consisting of two layers of cells: a superficial zone and a deep zone. The superficial zone contains ovoid, fusiform, or triangular, multipolar neurons. The deep zone has smaller, star-shaped cells with greatly branched processes, and also a larger type of cell similar to those found in the superficial zone. The axons of the larger cells of the deep zone and those of the superficial zone enter the tegmental region. The axons of the smaller cells of the deep zone, according to Cajal, break up into numerous arborizations in regions near the cell body. On entrance to the interpeduncular nucleus the fibers of the habenulo-peduncular tract swing across the nucleus to the opposite side of the brain, then curve back

again to break up ultimately in terminal arborizations on the side on which they enter. Fibers from the mammillary bodies also enter into the interpeduncular nucleus and constitute a mammillo-peduncular tract. Arising from the cells of the interpeduncular nucleus is the tractus pedunculo-tegmentalis. This tract terminates in the dorsal tegmental nucleus. The interpeduncular nucleus is a way station in the discharge of impulses from the hypothalamic olfacto-visceral and epithalamic olfacto-somatic correlation centers to the tegmental regions of the midbrain (Kappers). From the dorsal tegmental nucleus these impulses distribute by the dorsal longitudinal fasciculus of Schütz to the efferent centers of the brain stem (perhaps including the preganglionic centers such as the dorsal efferent nucleus of the vagus), thus constituting a way in which impulses from these regions may affect the autonomic nervous system (Kappers, '36; Huber, '26).

Nauta describes a paramedian region of the midbrain as the "limbic midbrain area". This is composed of the ventral tegmental area, the ventral half of the circumaqueductal gray substance and the reticular cell groups of Bechterew (nucleus reticularis tegmenti pontis) and Gudden (nucleus tegmentalis ventralis and dorsalis). The descending pathways, i.e. those projecting from the limbic forebrain to the subcortical area just mentioned are represented mainly by three fiber systems: (1) the fornix system, originating in the hippocampus, (2) the medial forebrain bundle, a multisynaptic fiber system of

heterogeneous origin traversing the lateral hypothalamic region and (3) a tract composed of the stria medullaris, habenula, and fasciculus retroflexus.

"An extensive mesencephalic region projects to the hypothalamus, preoptic area and medial septal nucleus, via the dorsal longitudinal fasciculus of Schütz and the system of the mammillary peduncle. The region of origin of these projections encompasses the ventral part of the periaqueductal gray substance, including Gudden's dorsal tegmental nucleus, and paramedian and medial tegmental cell groups, the deep tegmental nucleus and the ventral tegmental area of Tsai. It receives direct projections from spinal and trigeminal sensory cell groups, as well as ascending reticular projections. Together with the limbic system, *with which it is reciprocally connected, it appears to represent a neural mechanism of homeostatic control over endocrine and vegetative functions."[8]

* N.B. The broad term limbic system which, in addition to the cingulate and hippocampal gyri, the hippocampus, and the orbito-insulotemporal polar region, includes all the subcortical cell stations presumably associated with the 'limbic lobe'. These are the amygdala, septal nuclei, hypothalamus, epithalamus, anterior thalamic nuclei, and parts of the basal ganglia. Although the cortical areas contained in the 'limbic lobe' may share some structural characteristics, it is still not known whether they form a functional unit. (Kaada, 1960).

Problem Formulation

Investigators of the vertebrate habenula along with its fiber connections have suggested an olfacto-somatic reflex function. (Originally suggested by Edinger, 1911). This fiber system composed of the stria medullaris, habenula and fasciculus retroflexus has more recently been described as a reciprocal pathway projecting from the "limbic forebrain" to the "limbic midbrain area". (Nauta, '63).

Anatomical findings have consistently shown two distinct nuclei in the habenula, with fiber tracts to each coursing in the stria medullaris thalami. There is some controversy as to the origin and course of these tracts, especially when comparisons are made in different orders of mammals. However, better agreement has been found in the cat in studies in which newer silver impregnation techniques have been used. (Nauta, '56, '58, and Raisman, '66).

However, little attention has been placed on the anatomical course and distribution of the efferent fibers. Nauta, (cat, '58), reports a diffuse lateral tract arising from the fasciculus retroflexus and coursing through the parafascicular-centre median complex before turning caudally. Even more recently, Akagi ('67), reports differential connections to the mesencephalon from the medial and lateral habenular nucleus of the cat, but he does not describe the course of these fibers.

The purpose of this work is:

1. To describe anatomically the course and distribution of

the caudal projections of the habenula within the limbic midbrain area, using lesioned material stained for degenerating axons. More recent modifications of the Nauta ('54) and Heimer ('67) staining techniques will be used.

2. To study the morphology of the two nuclei in the habenula with the use of the Golgi technique and its modifications.

CHAPTER II

MATERIAL AND METHODS

Normal Studies *

The habenular nucleus of mice and cats was stained with modifications of the Golgi and Golgi-Cox methods. However, of the many modifications of Golgi's method, the Rapid Golgi, that is, dichromate-silver impregnation of brain tissue fixed by immersion in osmium tetroxide and dichromate salts, was found to be the most useful.

The brains of approximately 20 mice, 1-20 days of age were prepared by the Rapid method and cut in the three major planes of section. (Horizontal, sagittal, and frontal).

Different ages of cats were used depending on the modifications of the Golgi, 6-7 weeks for Golgi Fox and Kopsch, 2-14 days for Golgi Rapid. More than 30 brains of young cats were used in this study.

The Golgi Rapid gave some clear impregnations of cell bodies and dendritic ramifications in the habenula of cats 2-7 days old. Although the details of this method are well known,

* "Normal" refers to material in which no experimental lesions have been made.

some of the technical variables used are important. The length of fixation depends on species, age and size of tissue block. In general, the fixation times ranged from 24 hours to 14 days. Increasing periods of time in silver solution resulted in more extensive impregnation of neural elements, often at the expense of peripheral elements (medial nucleus) which showed heavy precipitation.

A schedule was worked out in which the two major variables of this technique could be evaluated as they pertain to the staining of the habenula. In one group the habenula of five kittens from the same litter was dissected out and blocks 2-3 mm. thick were fixed in 1% osmium-dichromate. The right habenula of each kitten was impregnated with silver for 24 hours, the left for 8 hours. The time of fixation prior to this impregnation was varied from 1-10 days. (Table I).

Table II shows another series of four kittens of the same litter, the right habenula fixed in osmium for 4 days, the left for 8 days. In this instance the time in silver was varied from 6-12 hours.

All these kittens were approximately nine days old and their eyes had not yet opened.

A choice must also be made regarding the age of the animals. The more mature the animal, the more closely the synaptic patterns approach that of the adult. However, maturization and myelinazation proceed simultaneously, and beyond 20-30 days, the average mammalian brain is too heavily myelinated to give

TABLE I.

O_sO_4 Days	$AgNO_3$ 24 hours	$AgNO_3$ 8 hours
1	O_{1R}	O_{2L}
2	O_{3R}	O_{4L}
4	O_{5R}	O_{6L}
6	O_{7R}	O_{8L}
10	O_{9R}	O_{10L}

TABLE II.

$AgNO_3$ Hours	O_sO_4 4 days	O_sO_4 8 days
6	S_{1R}	S_{2L}
8	S_{3R}	S_{4L}
10	S_{5R}	S_{6L}
12	S_{7R}	S_{8L}

good Golgi impregnations. New-born material gave the clearest impregnations but such material gives an immature version of the nervous system.

The tracing of axons over a large extent of the brain made the use of mice and/or rats desireable. Compared to cats, the number of neurons is still relatively small and the circuit systems less reduplicated. Unfortunately I was not able to obtain any impregnations of the habenula in mice or rats using any of the Golgi modifications.

Degeneration Studies

This report is based upon findings in 20 adult cats, 2.5-4 kgs. in weight, in which small electrolytic lesions had been placed in the habenular complex with the aid of a Kopf stereotaxic instrument. Electrocoagulation was carried out by means of a Stoelting Lesion Generating Device at 3 ma. for 10-30 seconds, using stainless steel electrodes. (26 gauge hypodermic needle tubing insulated with Epoxylite). The survival time varied between 5-8 days.

Three types of lesions were attempted:

A. in the medial habenula

B. in both medial and lateral habenula

C. in the stria medullaris thalami.

The lesion was made from a lateral approach in the contralateral habenula or from a horizontal approach in the ipsilateral habenula.

The animals were killed by a lethal of nembutal anesthetic and perfused with 200 cc. of physiological saline introduced in the left ventricle and followed by 250-300 cc. of 10% Formalin. The brains were removed and kept in 10% Formalin for 1-2 months before sectioning.

Frozen sections were cut at 30 mu., collected in 10% Formalin, then routinely stained with the Nauta-Laidlaw technique for degenerating axons, using either the phosphomolybdic or Uranyl Nitrate modification. Some sections were stained with the Fink-Heimer procedure II and counterstained with cresyl-echt violet.

CHAPTER III

OBSERVATIONS

Normal Studies

A. Technique:

Although several modifications of the Golgi technique were used, the most successful impregnations were obtained in young cats using the Golgi rapid method.

The results of the two important variables of this technique as they pertain to the staining of the habenula in the cat, i.e., the length of osmium-dichromate fixation and the time in silver solution are given in Tables III and IV.

Prolonged immersion in osmium-dichromate fixative (Table III) resulted in the staining of blood vessels and glial cells to the exclusion of any other elements. The blocks were also more difficult to section since they were very brittle. Short fixation times result in incomplete penetration of osmium. The optimal time for staining both the medial and lateral habenula is between two and four days, but good impregnations are obtained in specimens which were in the mordant up to six days.

The shorter periods of immersion in silver of 6-12 hours (Table III) as compared to the longer periods of 24 hours (Table IV) are more suited for staining the medial habenula. This decreases the amount of precipitation, but stains the axons very light brown

instead of the usual black color seen with longer impregnation times. The best immersion period was ten hours, providing impregnation of axons, dendrites, and somata of both divisions of the habenula. Unfortunately the eight day osmium time was too long and none of the blocks could be properly sectioned.

It can be concluded from the results presented in Tables III and IV that very short fixation time plus short impregnation time results in light precipitate but also a light brown color of the stained axons. (# O_2L, Table III). In most of these blocks there was incomplete penetration by the osmium indicated by the white color in the center of the block.

Long fixation time accompanied by long impregnation in silver (Table III, # O_9L), produces brittle sections and aggregations of precipitate throughout the section, especially in the peripheral area of the medial nucleus.

The best results are obtained from blocks in the mordant for four to six days (to ensure full penetration by osmium) followed by six to twelve hours of silver impregnation. Less time in silver can be allowed if only the medial nucleus is to be seen, since the apparently smaller elements are easily obscured by precipitates. Longer silver times were suitable for the lateral nucleus, which gave the clearest picture of all elements; axons, dendrites, somata, and even glial cells.

TABLE III. (Results).

O_sO_4 Days	$AgNO_3$ 24 hours	$AgNO_3$ 8 hours
1	O_{1R} Not completely fixed, but good impregnation of periphery. Light precipitate.	O_{2L} Not completely fixed in center, background dark, lateral habenular cells stained well.
2	O_{3R} Good impregnation of axons, spines seen on dendrites.	O_{4L} Very good impregnation of all elements, including some of medial nucleus.
4	O_{5R} Good impregnation of axons. Some precipitation in periphery.	O_{6L} Good impregnation of cell bodies, dendrites, and axons.
6	O_{7R} Good cells in lateral nucleus but most dendrites heavily precipitated.	O_{8L} Medial habenula nucleus, cell bodies, and dendrites stained well. No axons seen. Some difficulties in cutting.
10	O_{9R} Many blood vessels stained. Difficulty in cutting. Heavy precipitate.	O_{10L} Many blood vessels stained.

TABLE IV. (Results).

$AgNO_3$ Hours	O_sO_4 4 days	O_sO_4 8 days
6	S_{1R} Light impregnation, spines seen in lateral habenular nucleus. Good impregnation of axons.	S_{2L} Crumbled badly, could not cut.
8	S_{3R} Same as above.	S_{4L} Same as above.
10	S_{5R} Medial nucleus stained well. Cell bodies and dendrites. Lateral also nice, cell body, dendrites and axons stained.	S_{6L} Same as above.
12	S_{7R} Good impregnation of all elements.	S_{8L} Same as above.

B. Morphology:

Soma: Three types of neuron are seen in the Golgi preparations. Type 1 is found in the medial nucleus and is characterized by a small, round cell body ranging from 10-15 microns in diameter, (Fig. 1). Types 2 and 3 are found in the lateral nucleus and may be found in close association (Fig. 2). The soma of type 2 is round to fusiform in shape with 'projections' or spines seen with some frequency, (Fig. 3). These projections were never found on the perikaryon in the medial nucleus (type 1) or on type 3 which is pyramidal in shape. The greater diameter of types 2 and 3 is from 15-20 microns, some being as small as the largest soma (15 microns) of the medial nucleus.

Dendrite Patterns: The dendritic ramifications of type 1 are short with secondary or tertiary branching[9], (see Fig. 6B). Since only a few of this type of cell were stained it is not possible to determine any specific orientation of the branching pattern. The dendrites of both types in the lateral nucleus are diffusely branching, the secondary branch being longer than the primary, the tertiary longer than the secondary and oriented mainly in an antero-posterior direction. Some of the type 3 neuron are seen in frontal sections with a few basal dendritic

branches oriented in a medial and lateral direction. The dendritic trunks are sometimes quite large, one of each cell always being larger than the others, (see especially Fig. 2). Dendritic spines with bulbous endings were seldom seen but when present appeared on the secondary and tertiary branches of both cell types 2 and 3. (Fig. 4).

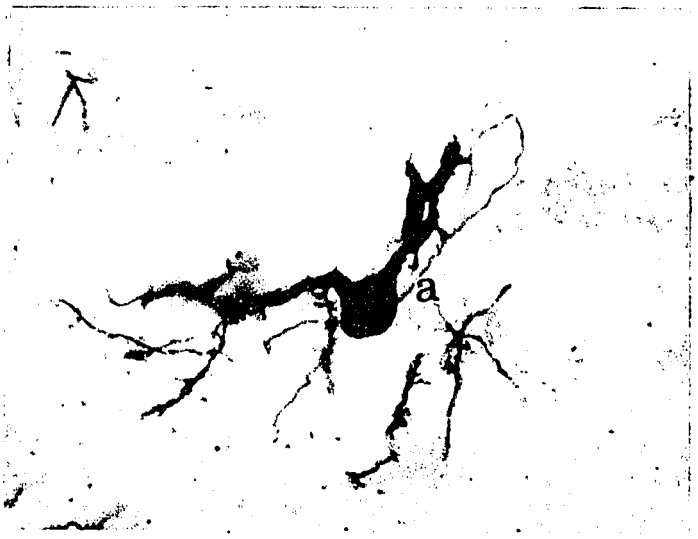
At times the dendrites of one cell could be seen coursing toward the dendrite of a neighboring cell, apparently making contact. (Fig. 5).

Axon Patterns: One axon of a cell in the medial nucleus (type 1) was seen. It is very thin, leaves the cell body and follows a winding path. (See a, Fig. 1). The axon of types 2 and 3 is of a small caliber and arises from the cell body or dendritic trunk, following a weavy path until descending into the fasciculus retroflexus. (See a, Fig 5 and a, Fig. 4). No axon collaterals are present.

Neuropil: The axons of the stria medullaris thalami appear as fine threads streaming ventrally through the habenula. The method of termination of these fine axons could not be determined. However, there was a specific pattern as to the mode of contact with the dendritic ramifications in the two nuclei.

In the medial nucleus the axons from the stria course in a parallel direction to the dendrites with marked swelling at the point of contiguity or crossing. (Fig. 6). In the lateral nucleus, the axons from the stria course in a direction at right angles to the dendritic ramifications. (Fig. 7).

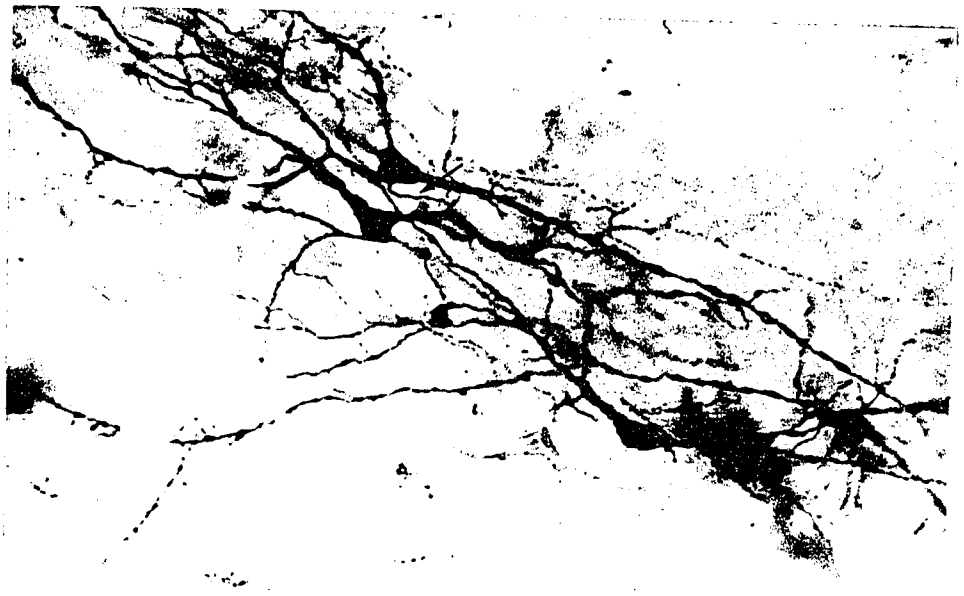
Glial fibrous astrocytes could also be readily identified in the neuropil. One such glial cell is seen in Figure 8.



25 microns

Figure 1: Cell of the posterior part of the medial habenular nucleus (type 1) in a 4 week old kitten. (a) indicates the axon arising from the cell body.

Sagittal section, mag. 390X, Golgi Kopsch.



70 microns

Figure 2: Grouping of cells in the lateral division of the habenula in a 9 day old kitten showing type 2 and 3. An axon of type 3 can be seen coursing posteriorly, arrow.

Sagittal section, mag. 280X, Golgi rapid.

Figure 3: 'Projections' or spines were observed with some frequency on the soma of the fusiform neuron in the lateral habenular nucleus. Some of these were found to have bulbous endings as seen above, arrow.

Frontal section, 9 day old kitten, mag. 1560X with water immersion lens, Golgi rapid.

Figure 4: Typical neurons of the lateral division of the habenular nucleus in a 9 day old kitten showing the two neuron types, pyramidal and fusiform. Arrow points out dendritic 'projections' or spines found on the secondary branch of the pyramidal cell. a, axon leaving dendritic trunk.

Sagittal section, mag. 900X, Golgi rapid.

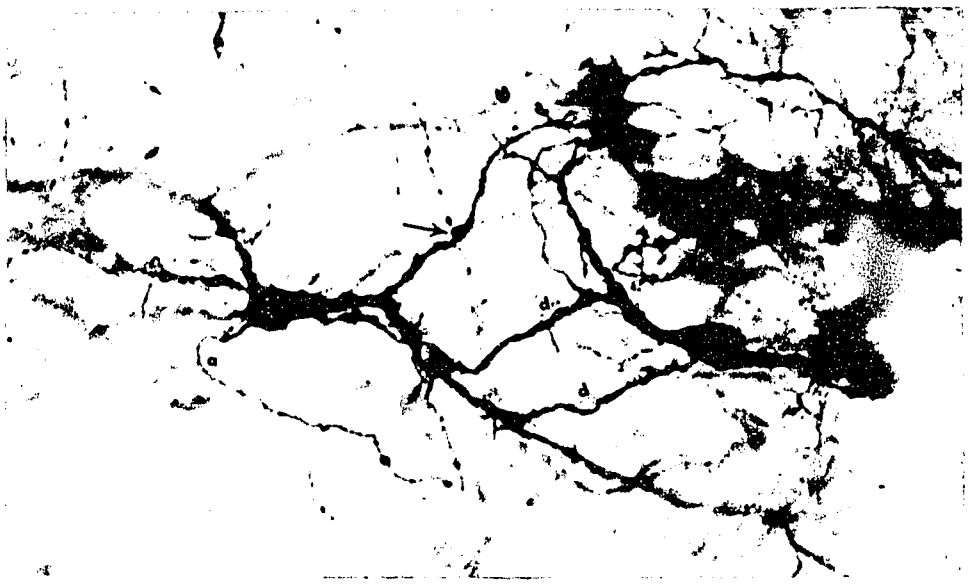


Figure 5: Two neurons of the lateral nucleus apparently making dendro-dendritic contact. One dendrite of each cell courses toward the other and cannot be followed further. d, dendrite, a, axon. Slight swellings can be seen at the point of contact, arrow, of passing axons.

Frontal section, 9 day old kitten, mag. 350X, Golgi rapid.

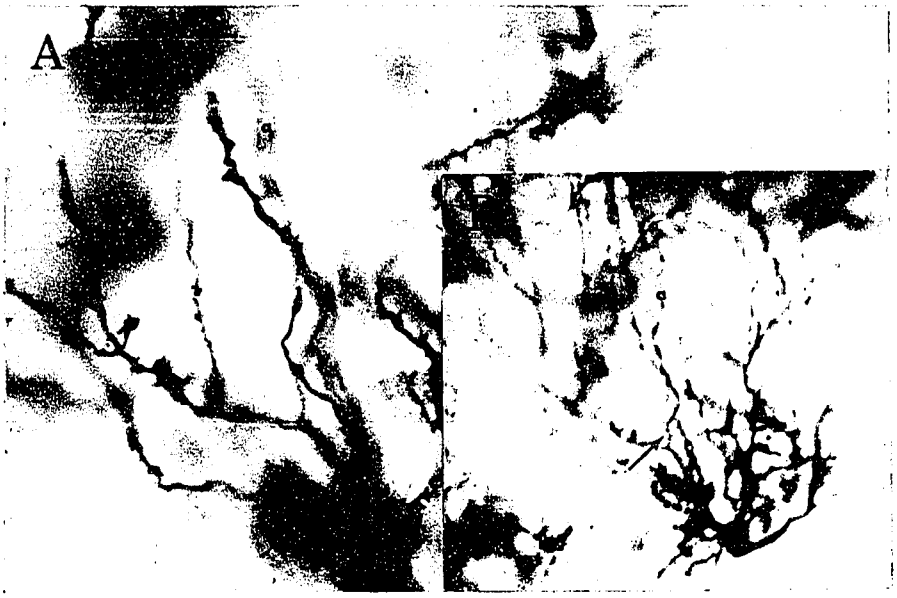


Figure 6: A. High magnification micrograph of afferent axon (a) coursing parallel to a dendrite of cell seen in part B, at arrow.

B. Cell of the medial habenular nucleus in a 9 day old kitten. The short branching pattern characteristic of type 2 is shown clearly in this micrograph.

Sagittal section, mag. 315X, Golgi rapid.

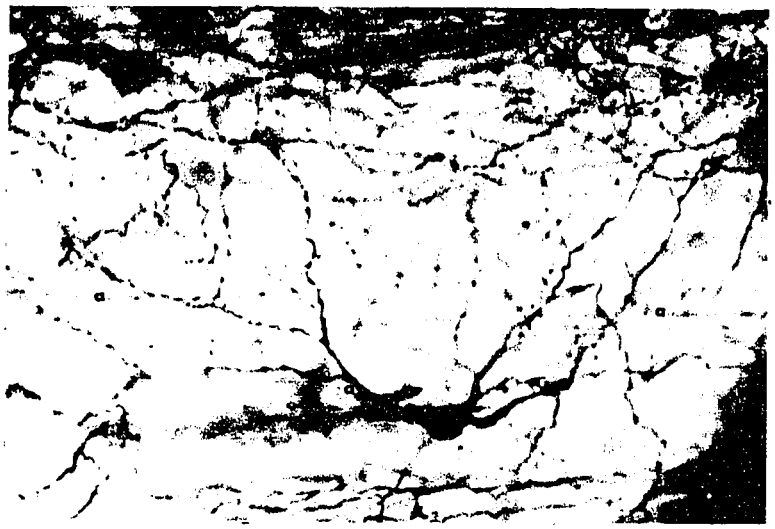


Figure 7: Cell of lateral habenula. Afferent axons (a) may be seen coursing at right angles to the dendritic arborization (d).

Sagittal section, 9 day old kitten, mag. 315X, Golgi rapid.

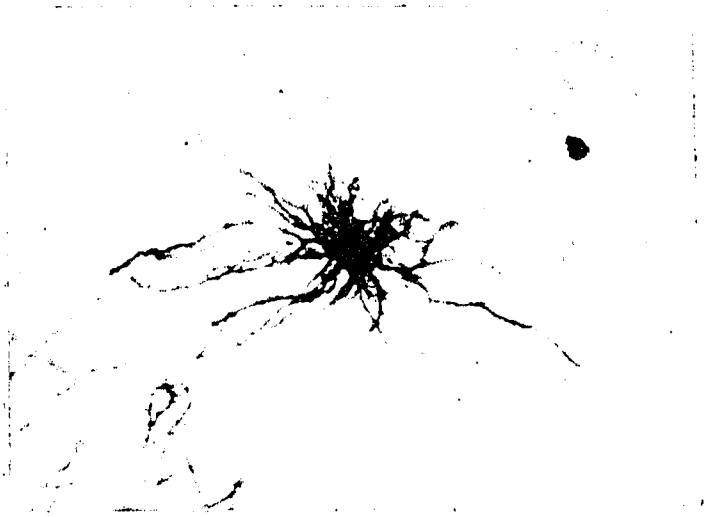


Figure 8: Glial astrocyte of the type found in both divisions of the habenula.

Sagittal section, mag. 390X, Golgi Kopsch.

DEGENERATION STUDIES

A. Lesion of the medial and lateral habenula: Cat number 13.

In this case, the lesion has been produced by an electrode introduced at an angle of 25 degrees from the contralateral side. It is confined mainly to the habenular nucleus on the left side but a very small portion reached the posterior medial-dorsal nucleus of the thalamus as indicated by figure 9. Only the fiber degeneration extending caudalward from the lesion will be described.

As shown by Fig. 10, two main tracts of degenerated fibers can be followed from this lesion. The first is incorporated in the fasciculus retroflexus, (indicated in Fig. 10A by the highest density of dots just lateral to the paraventricular nucleus). A moderate amount of degeneration is seen throughout the fasciculus retroflexus as it courses ventrally and caudally, through the rostromedial part of the red nucleus. A diffuse system courses laterally and caudally from the posterior one-third of the habenula passing through the centre-median nucleus and mesencephalic reticular formation. Very few degenerated terminals are seen in the centre-median nucleus, and only fibers of passage are in the mesencephalic reticular formation. These "fibers of passage" gradually turn medialward above the red nucleus to join the compact fasciculus retroflexus on its lateral side. Figure 11A shows a portion of this diffuse tract as it

passes through the centre-median nucleus. Only a few scattered terminal endings are indicated. Figure 11B is a micrograph at the same magnification showing the fibers "en passant" joining the fasciculus retroflexus, medial to the red nucleus. Both compact (medial) and diffuse (lateral) parts of the fasciculus then continue ventralward (Fig. 10C), lateral to the interpeduncular nucleus, forming (in frontal sections) a triangular area of heavily degenerated fibers and terminals, Fig. 12. The more medially placed fibers turn into the interpeduncular nucleus traversing from left to right, Fig. 13. The heaviest terminal degeneration occurs in the lateral portion of the interpeduncular nucleus, more distinct on the lesion side. The more laterally placed fibers and a few which have crossed in the interpeduncular nucleus gradually turn dorsalward in the midline of the upper midbrain tegmentum (Fig. 10D and E), some ending in the praedorsal area, (Fig. 14), and nucleus centralis tegmenti superior, (Fig. 15). A few finer fibers then continue to the nucleus dorsalis tegmenti profundus and nucleus dorsalis tegmenti of Von Gudden (see Fig. 15) and finally to the caudal part of the central gray substance, (Fig. 16). (Terminology of brain stem structures according to Verhaart Atlas, '64).

A considerable number of degenerated axons from the habenula leave the fasciculus retroflexus medially to terminate in the paraventricular and parafascicular nuclei, Figures 17 and 18. (The parafascicular nucleus is defined as that part of the parafascicular-centre median complex lying medial to

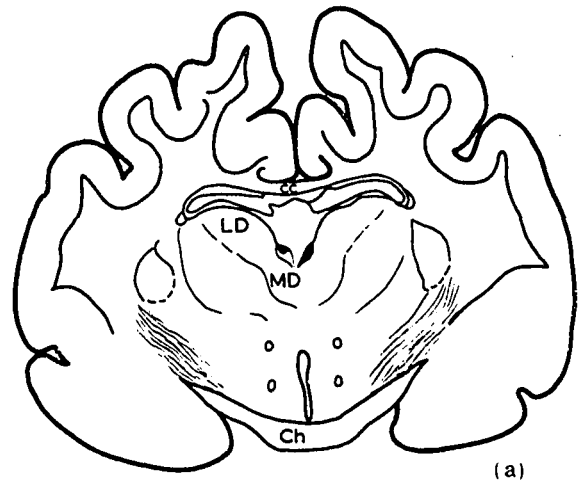
the fasciculus retroflexus).

Figure 9: Projection drawings of the habenula lesion in cat number 13.

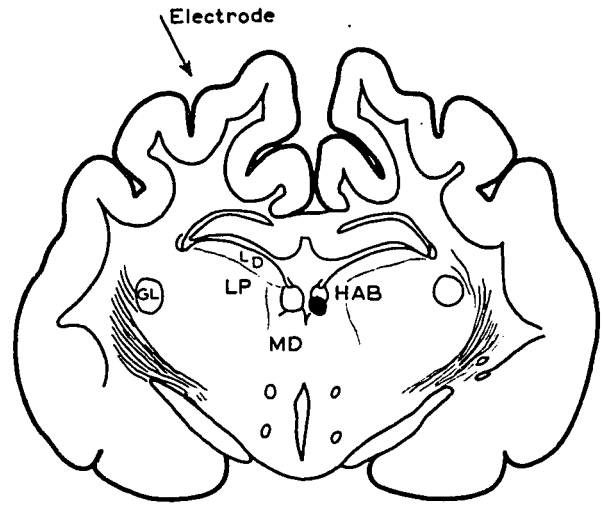
Drawing (a) shows the most rostral extent confined to the habenula and stria medullaris thalami.

Drawing (b) is the largest area of damage to the medial and lateral habenula. A small portion of the paraventricular and dorsomedial nuclei is also lesioned.

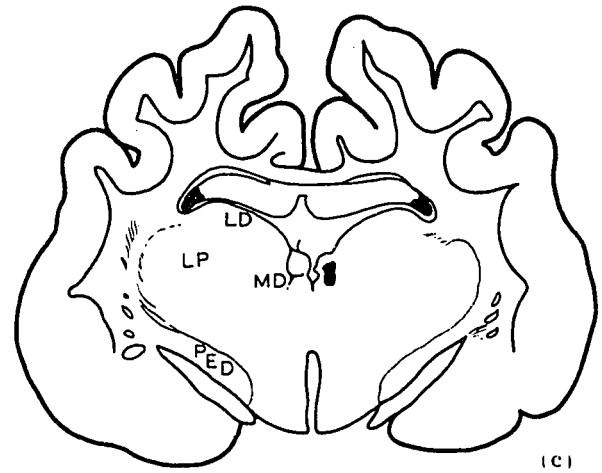
Drawing (c) indicates the caudal extent with damage to the lateral habenula and a small part of the dorsomedial nucleus.



(a)



(b)

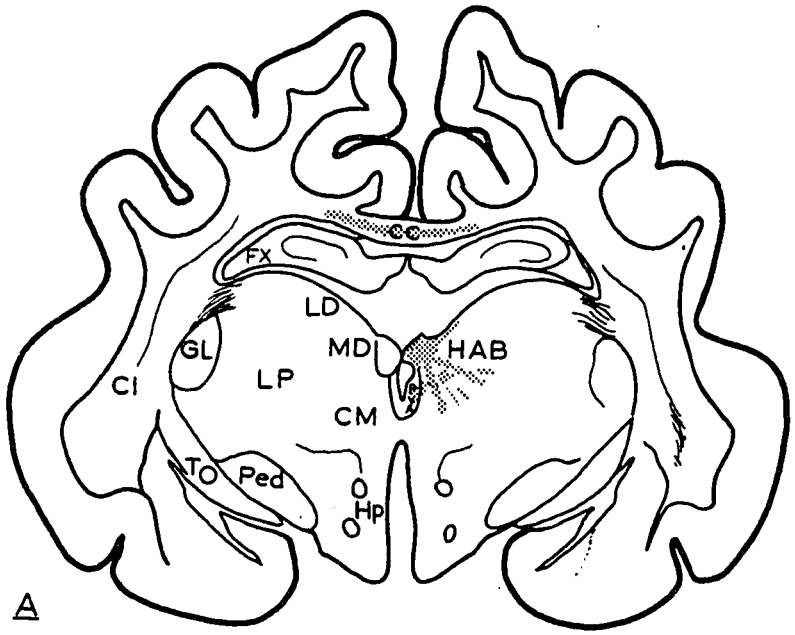


(c)

CAT 13

Figure 10: Projection drawings of selected Nauta and Fink-Heimer sections from Cat number 13. The course dots indicate degenerated fibers, terminals are described in the text. Oblique frontal plane.

Degeneration is followed in the fasciculus retroflexus, centra-median nucleus and mesencephalic reticular formation. Most of these fibers descend to the interpeduncular and ventral tegmental nuclei. Longer fibers enter the midbrain tegmentum to distribute to the nucleus centralis tegmenti, praedorsal fascicle, tegmental nuclei of Von Gudden and central grey.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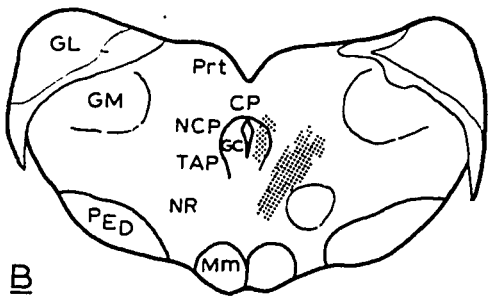
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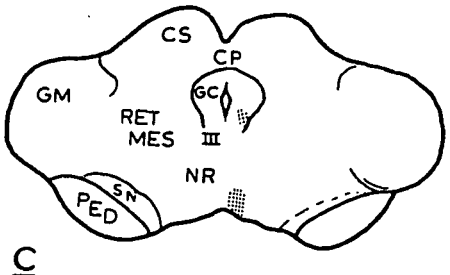
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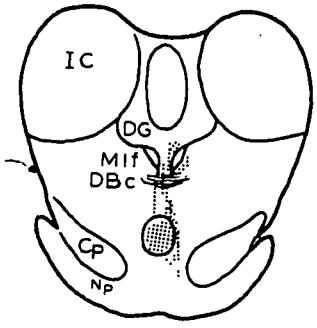
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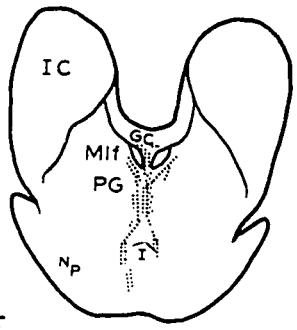
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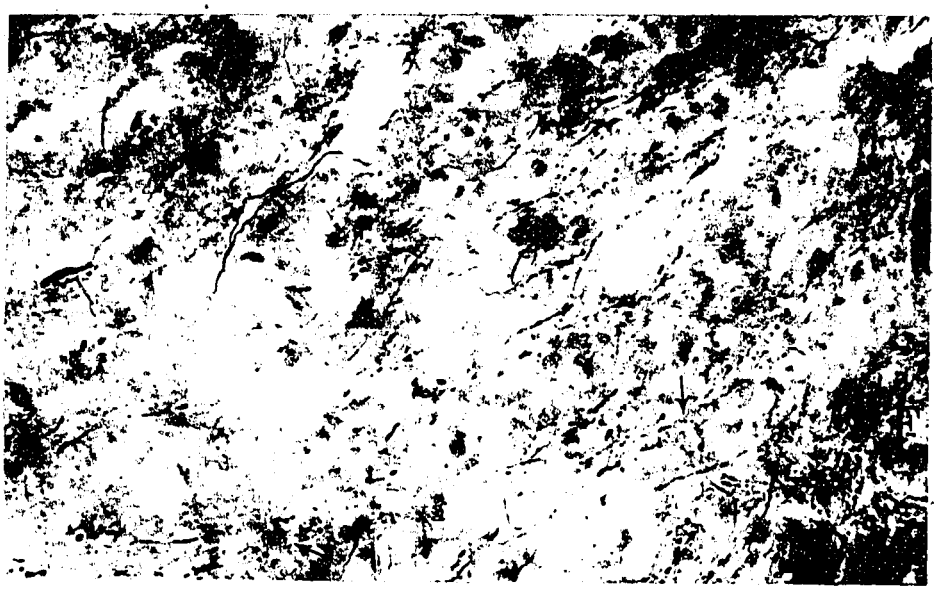


Figure 11A: Degenerated fibers coursing through the centre median nucleus, arrows, terminal endings.
F, fasciculus retroflexus.

Cat 13, Fink-Heimer Procedure II. Oblique frontal section, mag. 240X.

Figure 11B: Degenerated fibers coursing through the mesencephalic reticular formation.
F, fasciculus retroflexus.

Cat 13, Fink-Heimer Procedure II. Oblique frontal section, mag. 240X.

Figure 12: Heavily degenerated fibers and terminals in the ventral tegmental area of Tsai, (see also Fig. 20C; Cat 10).

Cat 13, frontal plane, Fink-Heimer Procedure II, Mag. 680X.

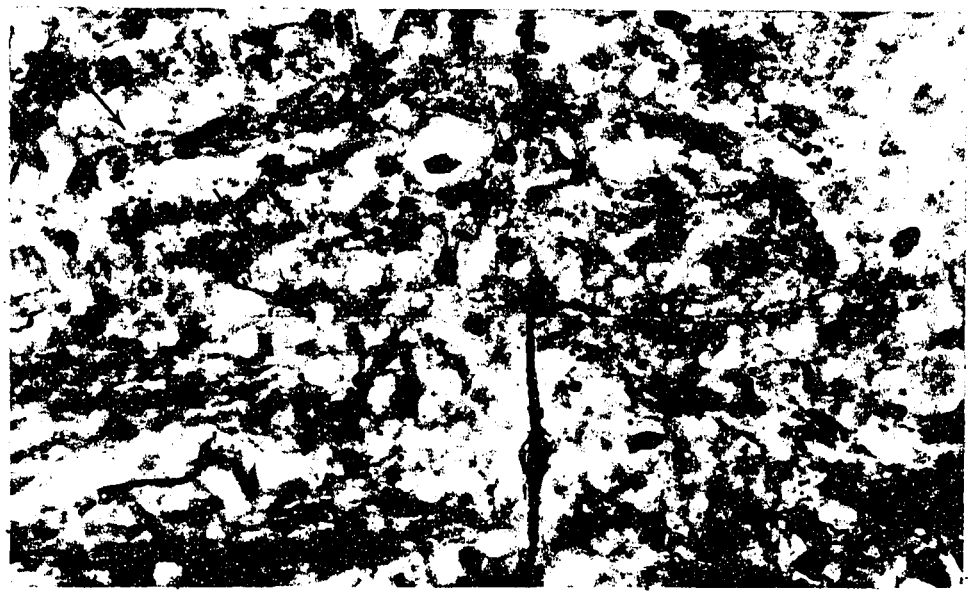


Figure 13: Degenerated fibers and terminals in the caudal and medial interpeduncular nucleus. Arrows indicate terminal endings. (See also Fig. 10D).

Cat 13, frontal plane, Fink-Heimer Procedure II, mag. 640X.

medial lateral

Figure 14: Terminal degeneration in the praedorsal area at the level of the inferior colliculus. (See Fig. 10E).

Cat 13, Fink-Heimer Procedure II, frontal section, mag. 625X.

Figure 15: Degenerated fibers and terminals in the dorsal raphe of the midbrain tegmentum and nucleus dorsalis tegmenti profundi. (See Figure 10, D and E).

NCS, nucleus centralis tegmenti superior (Bechterew),
PG, nucleus dorsalis profundi,
Mlf, medial longitudinal fasciculus.

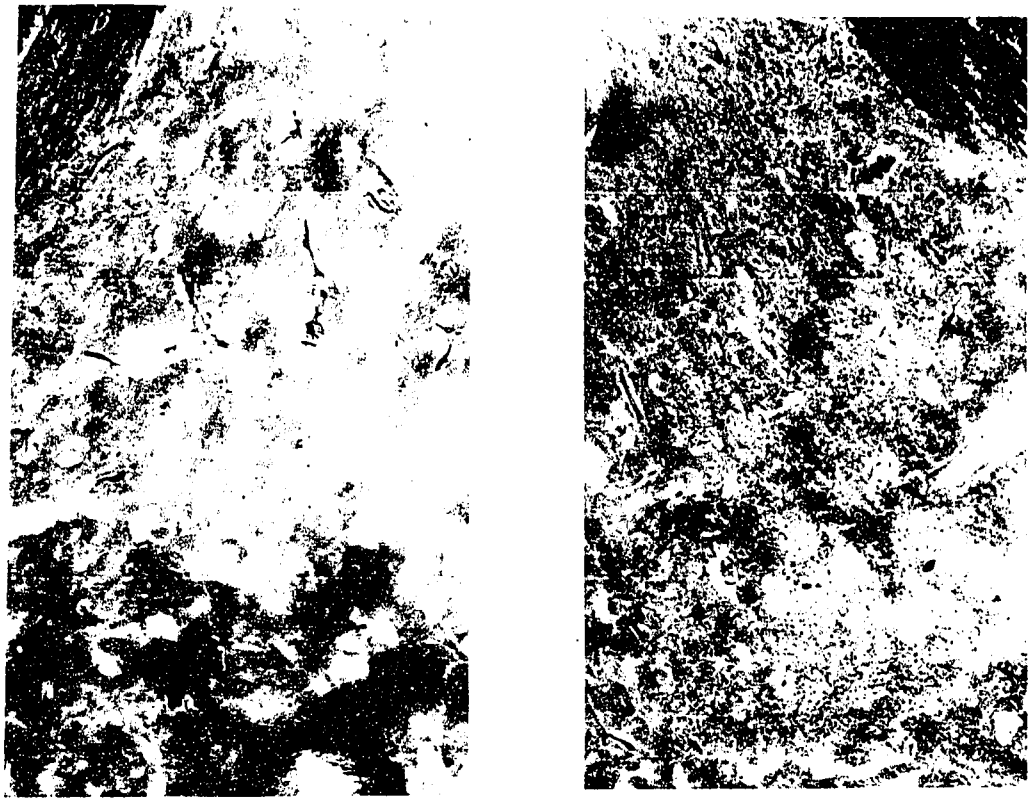
Cat 13, Fink-Heimer Procedure II, frontal section, mag. 250X.

Figure 16: Light fiber and terminal degeneration in the caudal central grey. (See Fig. 10E).

Cat 13, Fink-Heimer Procedure II, frontal section, mag. 450X.

Figure 17: Degeneration in the paraventricular area.

Cat 13, frontal plane, Fink-Heimer Procedure II, mag. 375X.



A_1 Control Side A_2 Lesion Side

Figure 18A: Fiber and terminal degeneration in the parafascicular area. The contralateral side is normal. Note marked degeneration in the fasciculus retroflexus in A_2.

Cat 13, Fink-Heimer Procedure II, oblique frontal plane, mag. 150X.

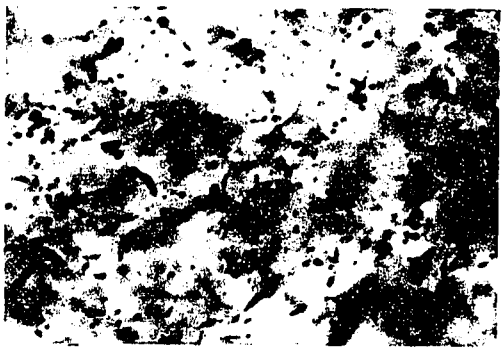


Figure 18B: Higher magnification (750X) of A_2 demonstrating a circular distribution of degenerated terminals around the cell body.

B. Lesion of the medial and lateral habenula: Cat number 10.

The lesioning electrode was introduced at Anterior 7.0, Lateral 1.0, and Horizontal plus 4.0 (Jasper, '54), on the ipsilateral side. As can be seen in Fig. 19, this lesion destroyed the entire habenular complex, extending anteriorly into the dorso-medial and paraventricular nuclei and posteriorly to the pretectal area, posterior commissure, and commissure of the superior colliculus.

These sections are more difficult to interpret because of the large lesion and the ipsilateral approach. They confirm the course of the two major pathways from the habenula (Fig. 20A). Figure 20, B and C, show very well the course of the fasciculus retroflexus as it appears between the fibers of the third nerve and then lateral to the interpeduncular nucleus. The heaviest degeneration in the interpeduncular nucleus occurs at the level of the inferior colliculus. This brain was stained using the Nauta method and only degenerated fibers are seen in the interpeduncular nucleus. The area possibly indicating terminals is at the lateral extreme of the nucleus.

From the lesion in the dorsomedial nucleus, degeneration is seen in this nucleus, in a small part of the nucleus lateralis dorsalis and in the contralateral centre-median nucleus. Because of the posterior extent of the lesion, degenerating axons are found in the pretectal area, superior colliculus, posterior commissure and nucleus of the posterior commissure. Degenerated

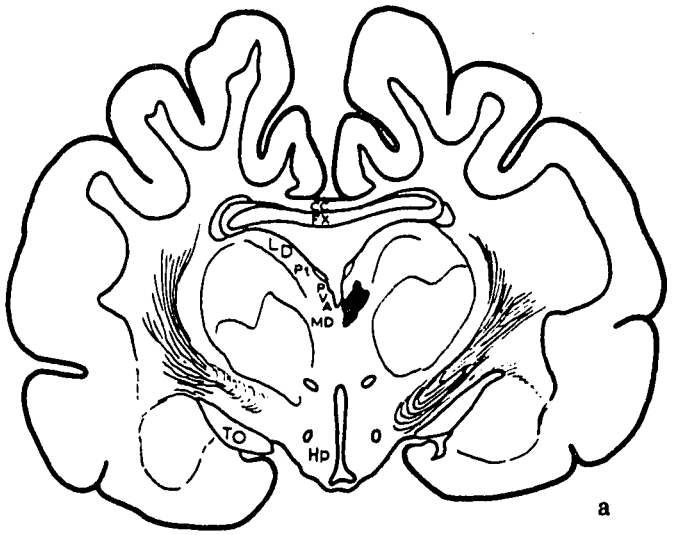
fibers are also in the contralateral stria medullaris. (See Figure 20, A to D). No degeneration is seen in the paraventricular or parafascicular nuclei.

Figure 19: Projection drawings of the habenula lesion in cat number 10.

Drawing (a) is anterior to the level of the habenula and shows a moderate amount of damage in the paraventricular and mediodorsal nuclei.

In (b), the entire left habenula is destroyed and also the stria medullaris at this level.

Drawing (c) shows the caudal extent involving the posterior commissure and pretectal area.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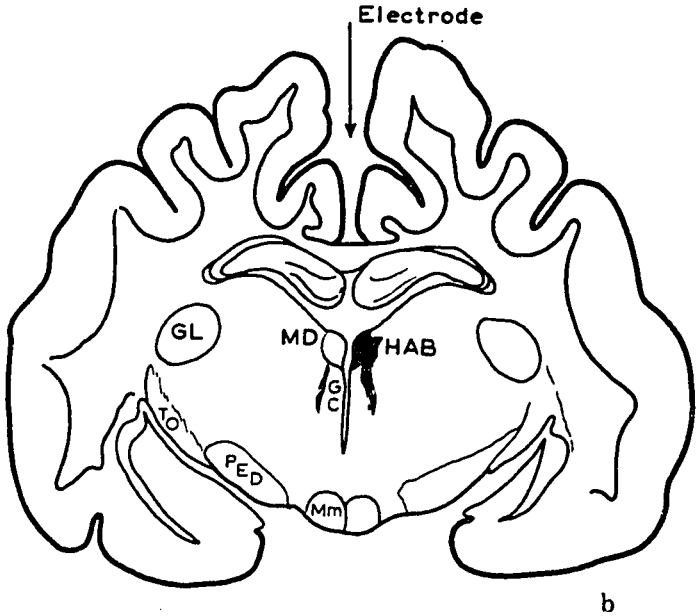
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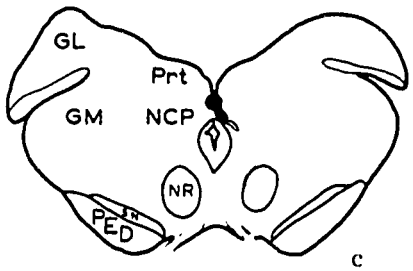
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CAT 10

Figure 20: Projection drawings of selected Nauta sections, cat number 10, demonstrating the course and destination of fibers from the habenula. Oblique frontal sections.

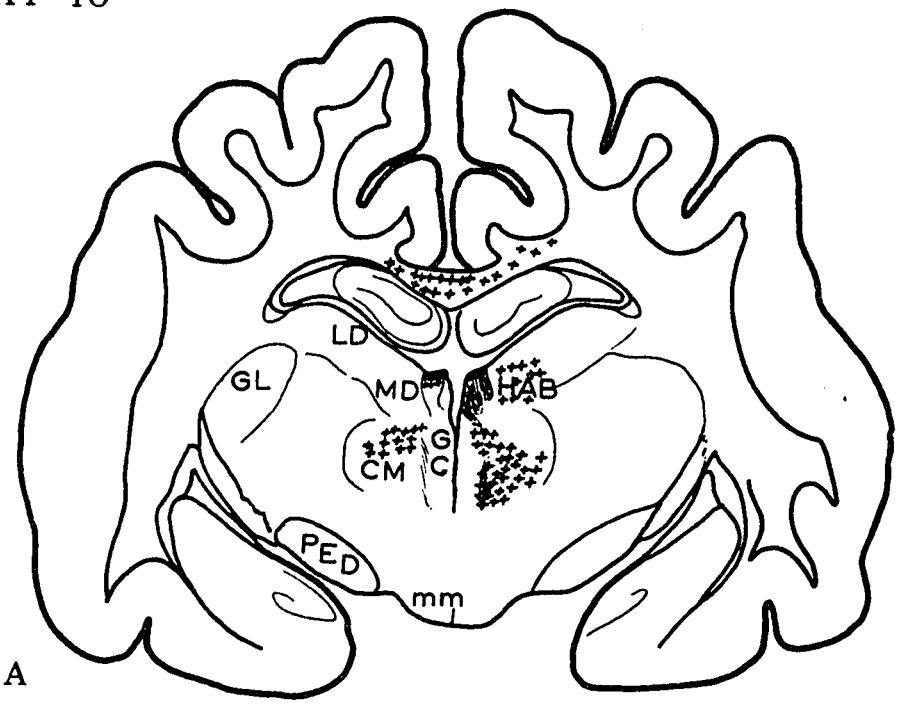
A: Heavy degeneration, (crosses), is seen in the fasciculus retroflexus and centre median nucleus. Contralateral to the side of the lesion, degenerated fibers are seen in the stria medullaris and centre median nucleus.

B: Degeneration extending from the immediate area of the lesion is seen dorsally. Ventrally the degenerated fasciculus retroplexus is descending between the fibers of the IIIrd nerve.

C: The fasciculus retroplexus forms an area of heavy degeneration just lateral to the interpeduncular nucleus. This area is described as the ventral tegmental area of Tsai and is depicted in cat number 13, Fig. 12.

D: Depicts heavy degeneration in the interpeduncular nucleus at the level of the inferior colliculus with longer fibers terminating in the nucleus dorsalis tegmenti profundi.

CAT IO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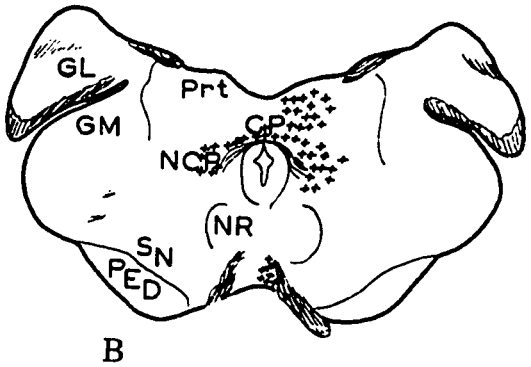
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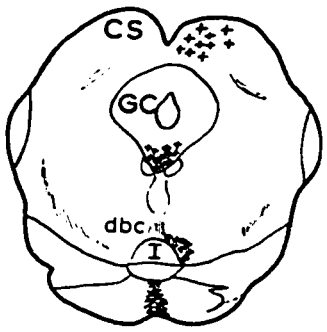
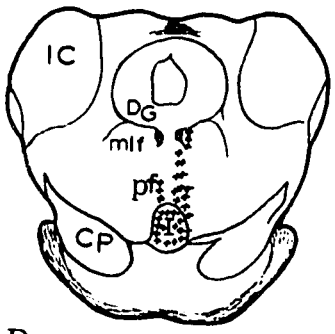
B


CS
GC
dbc
I

C


IC
DG
mlf
pf
CP

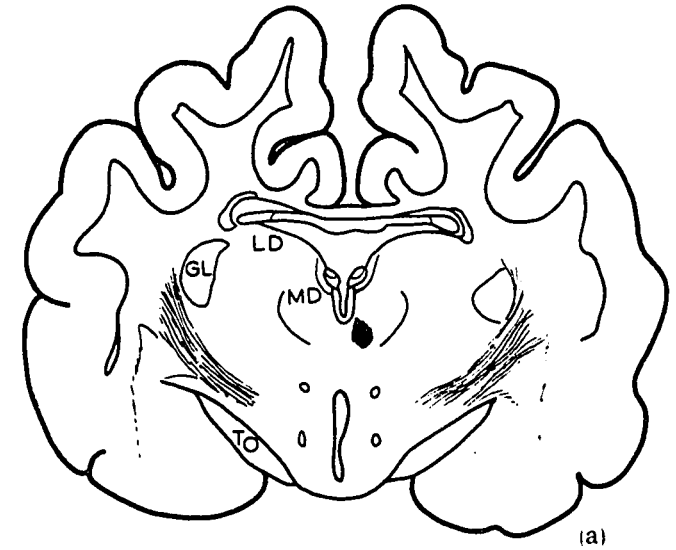
D

C. Lesion of the lateral habenular nucleus and stria medullaris: Cat number 9.

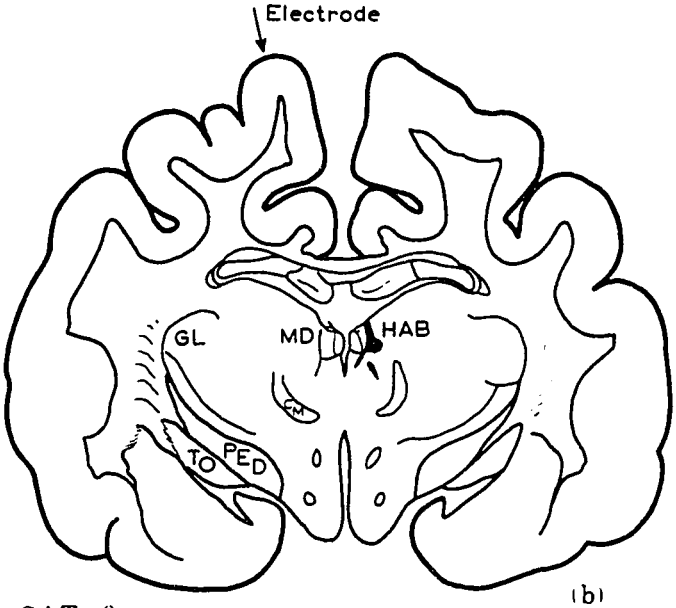
This lesion has been made by an electrode introduced at an angle of approximately 15 degrees from the contralateral side. Figure 21 shows that this lesion extended into the medial dorsal nucleus and periventricular grey.

Although some difficulty was experienced in the staining of this brain, these results, coincide with those found in cats 13 and 10. No degeneration is seen on the contralateral side except in the fornix.

Figure 21: a and b indicate the extent of a lesion directed to the lateral habenula in cat number 9. Damage is seen a) anterior to the habenula in the mediodorsal nucleus and b) in the lateral habenula and stria medullaris.



(a)



(b)

CAT 9

D. Lesion of the medial habenular nucleus: Cat number 11 and 19.

In these cats, the electrode was introduced on the ipsilateral side about 0.5 mm from the midline. In Cat 11, the electrode did not damage any other structure except along the electrode path, i.e., fornix and corpus callosum. Cat number 11 was sectioned in a frontal plane, number 19 in a sagittal plane, (Fig. 22).

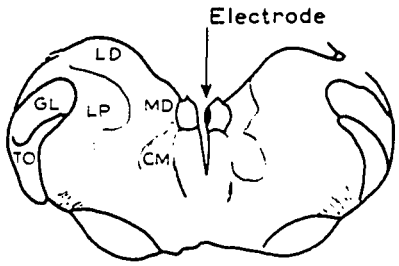
Degenerated axons are seen in the compact fasciculus retroflexus, but the more diffuse lateral fiber system is entirely normal, (Fig. 23). This suggests that the origin of the diffuse projection is limited to the lateral habenular nucleus or stria medullaris. The compact bundle passes backward and downward to the level of the interpeduncular nucleus where it forms a small area of moderate degeneration just lateral to the nucleus, the ventral tegmental area of Tsai. Fibers from the ventral tegmental area terminate in the ventral interpeduncular nucleus, (Fig. 24).

No degenerated axons are seen coursing to the parafascicular, paraventricular, or contralateral habenular nuclei. The interhabenular commissure was also completely normal.

Figure 22:

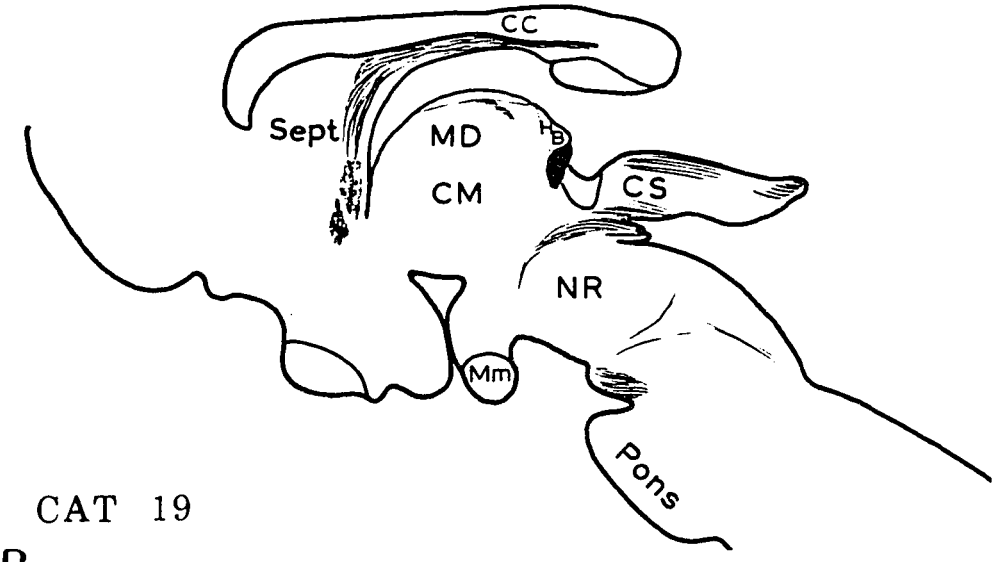
A: Projection drawing of the lesion of the caudal portion in the medial habenula nucleus, cat number 11, frontal plane.

B: Projection drawing of the lesion in the medial habenular nucleus, cat number 19, segittal plane. A small portion of the habenula and posterior commissure is also involved.



CAT 11

A



CAT 19

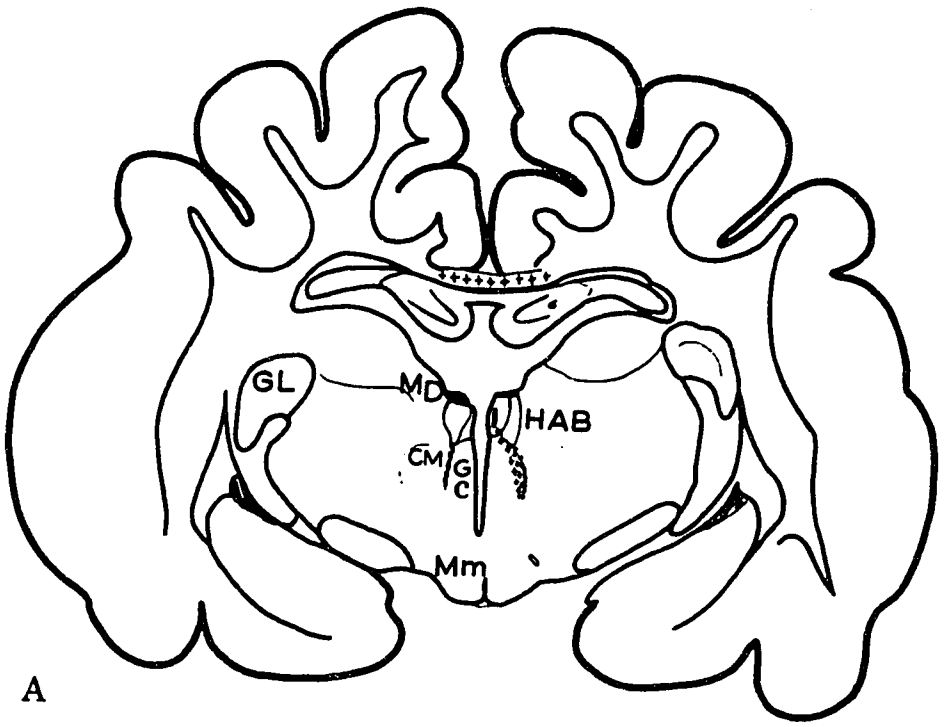
B

Figure 23: Nauta stain of the fasciculus retroflexus showing degenerated fibers in the medial portion from a lesion in the medial habenular nucleus.

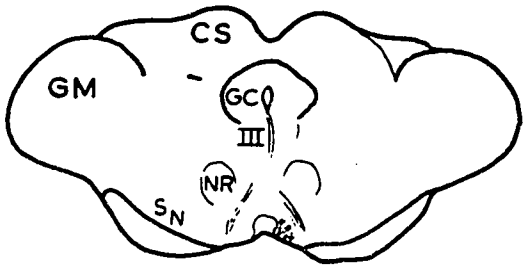
Cat 11, Nauta phosphomolybdic, frontal plane, mag. 438X.

Figure 24: Fiber degeneration following a lesion restricted to the medial division of the left habenula. The caudal part of the lesion is indicated in black in Fig. A. Small crosses indicate course of degenerated fibers. No degeneration is found beyond the level of the caudal interpeduncular nucleus.

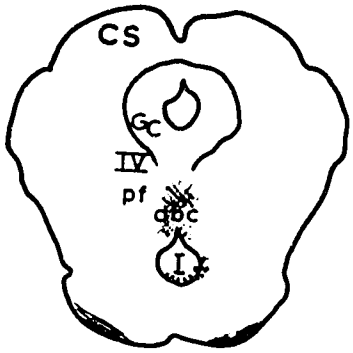
CAT II



A



B



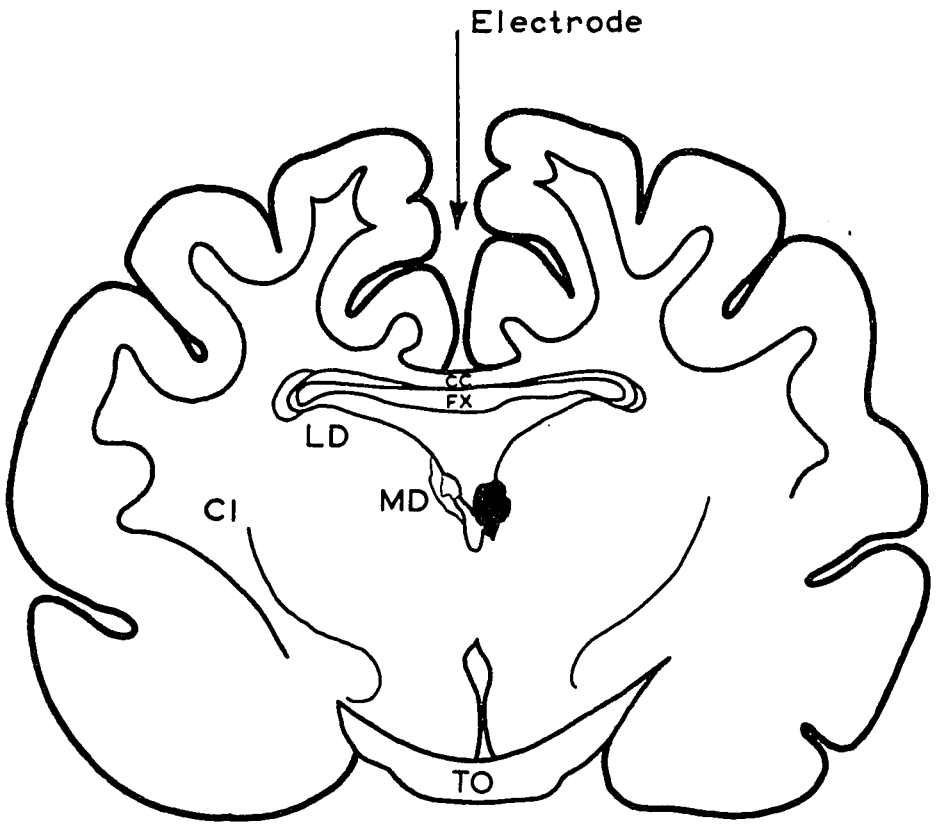
C

E. Lesion of the stria medullaris thalami: Cat number 20. (control).

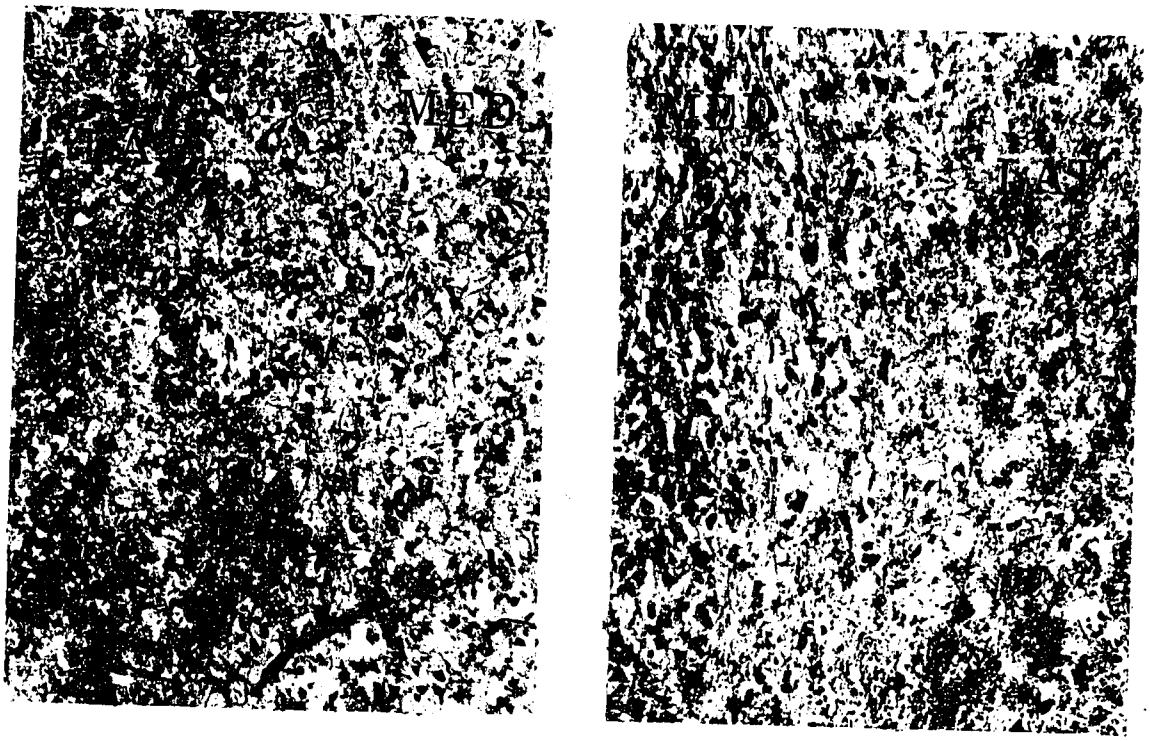
The path of this electrode is ipsilateral to the lesion approximately 0.5 mm from the midline. The lesion is about 2 mm in diameter, 2 mm in an anteroposterior direction, involving the stria and also the paraventricular and dorsal medial nuclei, Fig. 25.

On the side of the lesion, degenerating axons can be followed posteriorly in the stria medullaris passing between the medial and lateral nuclei of the habenula to descend a short distance in the fasciculus retroflexus. After traveling about 1 mm in the fasciculus retroflexus these fibers course medially into the parafascicular nucleus where heavy terminal degeneration is seen. There are also degenerating terminals in the lateral and medial habenula and contralateral, lateral habenular, (Fig. 26). As well, degenerating fibers are seen in the contralateral stria medullaris. No degeneration is seen in the fasciculus retroflexus beyond the level of the parafascicular nucleus or in the centre-median nucleus.

Figure 25: Projection drawing of the stria medullaris thalami lesion showing the most rostral extent of damage. This lesion extended 2mm caudalward and encroaches upon the paraventricular, parataenial and dorso-medial nuclei.



CAT 20



Contralateral to Lesion

Ipsilateral to Lesion

Figure 26: Degenerated fibers and terminals in the right and left habenula from a lesion in the left stria medullaris thalami. Note that the medial habenula nucleus on the side contralateral to the lesion has very little degeneration.

MED - Medial habenular nucleus.
LAT - Lateral habenular nucleus.

Cat 20, Fink-Heimer Procedure II, frontal plane, mag. 270X.

TEXT ABBREVIATIONS

CC	-	Corpus collosum.
CL	-	Claustrum.
CM	-	N. centrum medianum.
CP	-	Commissura posterior.
CS	-	Colliculus superior.
DBc	-	Decussatio brachiorum conguntivorum.
DG	-	N. dorsalis tegmenti of Von Gudden.
FX	-	Fornix.
GC	-	Griseum centrale.
GL	-	Corpus geniculatum laterale.
GM	-	Corpus geniculatum mediale.
HAB	-	N. habenularis.
Hp	-	Hypothalamus posterior.
I	-	N. interpeduncularis.
IC	-	Inferior colliculus.
LD	-	N. lateralis dorsalis.
LP	-	N. lateralis posterior.
MD	-	N. medialis dorsalis.
Mlf	-	Medial longitudinal fasicle.
Mm	-	Corpus mamillare.
NCP	-	N. commissurae posterioris.
NP	-	Nuclei pontis basales.
NR	-	N. ruber.

Ped	-	Pedunculus cerebralis.
Pf	-	N. parafascicularis.
pf	-	Predorsal fascicle.
PG	-	N. dorsalis tegmenti profundus of Von Gudden.
Prt	-	Praetectum.
PVA	-	N. periventricularis anterior.
RET. MES.	-	Substantia reticularis mesencephalica.
THP	-	Tractus habenulo-peduncularis.
TO	-	Tractus opticus.
III	-	Third nerve.

CHAPTER IV

DISCUSSION

The importance currently placed on the stria medullaris, habenula, and fasciculus retroflexus as a descending tract in the limbic system requires more precise knowledge of the fine structure of the habenula, a terminal nucleus for many fibers of diverse origin, and of its related fiber tracts, especially those to the midbrain.

It is customary in the morphological literature to subdivide the habenula into two distinct divisions, a medial and a lateral. However, some authors (Gurdjian, Tsai, Papez, Bodian, Marburg, to mention a few) have subdivided these two divisions even further. The first division into a medial and a lateral nucleus is based on distinct cell types and staining of nissl substance but any further subdividing must be done on the basis of cell clumping or grouping. The groups I and II of Marburg (human material) are parts of the nucleus habenularis medialis while groups IV and V, and possibly also the indistinct group III, belong to the nucleus habenularis lateralis.

In the cat, we have observed two distinct divisions, based on cell size and dendritic ramifications, in Golgi preparations. The cells of the medial nucleus are small, rounded cells with short, multibranching dendrites, confirming the description of Cajal for the dog. The dendritic ramification

can be included in the specialized dendritic pattern as described by Moliner ('62), which he found to be present in primary or secondary sensory relay centers characterized by afferent connections of homogenous origin. The cells of the lateral nucleus are round to fusiform and triangular with a radiate dendritic branching pattern. Moliner has related this dendritic pattern to an input of heterogenous origin and/or to the presence of relatively widely spaced afferent terminal fibers.

As described in the Literature Review, fibers to the lateral habenular nucleus arise from the lateral preoptic area (cat), pallidum (monkey), lateral hypothalamic area (Knook, rat), interpeduncular nucleus (cat), and interpeduncular commissure (our studies, cat). Afferents exclusively to the medial nucleus have been described from the septal area (cat). Thus, the medial nucleus does appear to have an input of homogeneous origin. The very diffuse dendritic branching pattern in the lateral nucleus can be correlated with an input of widely spaced and heterogenous origin.

Another distinction between the morphology of the medial and lateral nuclei of the habenula can be made on the basis of the course of afferents from the stria medullaris in relation to the dendritic branching pattern in the two nuclei. Our observations in Golgi sections reveal axons from the stria medullaris running downwards, parallel to the dendrites in the medial habenula. In contrast, long axons crossing the dendrites at right angles are seen in the lateral habenula. It would seem that the influence of the afferents on the nuclei in the medial

division would be confined to only a few neurons, whereas axons coursing in the lateral division would have their influence on many neurons along a greater area.

Leontovich ('63) has made a distinction between the structure of neurons of the reticular formation and neurons from specific structures of the brain and uses this histological criterion for assigning the nuclei of the lateral habenula, parafascicular, reticularis thalami, and others to the reticular system. He describes the reticular neurons as cells with only a few long, straight, little ramified dendrites. The reticular cells differ from the neurons of specific formation, both sensory and motor, which are characterized by numerous dendrites with dense, winding (bushy) ramification. Using this concept Leontovich describes the reticular system as a centrally located uninterrupted cell column throughout the spinal cord and brain stem which extends to the basal regions of the diencephalon and the telencephalon. This author shows drawings of a Golgi stain of the lateral habenula in the dog with poorly ramified dendrites. Our Golgi studies in the cat show more extensive branching with longer fibers. Nevertheless, they fall within the morphological description of a "reticular system" neuron.

Cajal has described the axons of the lateral nucleus as giving off three or more collaterals. No collaterals were seen in the present study although this may be due to the technique used. It has been shown by some authors (Tömbol, '66) that double and triple impregnations result in good staining of collateral axons in the thalamic nuclei. Since only single impregnations

are used in this study, the impregnation was not as complete as it might have been.

In our Golgi studies the dendrites of the lateral nucleus occassionaly show long, thin and pointed processes which are probably not fully stained boutons terminaux terminating on the dendrites. Another type of dendritic projection with a bulbous ending is also found on these neurons. The existence of such spine-like projections in the habenula has been confirmed with the electron microscope by Milhaud and Pappas, ('66).

Somatic projections or spines have not been described before in the habenula. Takahashi ('67) has described an axo-somatic ciliary ganglion synapse in the chick with electron microscopic sections and finds the morphological characteristics similar to that found in the habenula and interpeduncular nuclei of the cat, (Milhaud and Pappas, '66). Mugnaini, ('67), in Golgi preparations, reports spine-like structures on the cell soma of the lateral vestibular nucleus in the cat like those seen by us in the habenula. He has described the spines as being of a simple type with a short neck of varying diameter and often, but not always, with a small head. He has also confirmed the presence of these spines with the electronmicro-scope. Golgi studies have shown that the same situation exists in the other vestibular nuclei (Hauglie-Hanssen, '68) and electron microscopical observations in the nucleus cervicalis lateralis of the cat, (Westman and Grant, '65) and in the cuneate nucleus of the cat (Walberg, '66).

Cajal was one of the first to advance an opinion about

the possible functional significance of the dendritic spines. He reported (1896) that the main function of the spines must be to increase the receptive surface of the dendrite or to facilitate better contacts between the dendrites and the adjacent axons. In 1959, Gray presented the first electron microscopic evidence proving that the spines were the site of synaptic contact.

The axon of the three types of cells found in the habenula could not be traced very far from their origin in the Golgi sections. However, a clear picture of the course and termination of these axons was obtained by the Nauta, and Fink-Heimer stains for degenerating axons.

As shown by our results, the medial nucleus projects mainly to the interpeduncular nucleus via the compact fasciculus retroflexus. The electrode path of the control lesion in the stria medullaris passes through the same structure as the lesion in the medial habenula nucleus, i.e., the cortex, corpus callosum, and the fornix. Since no degeneration from these structures is seen beyond the parafascicular area, there can be no doubt that the degenerated fibers in the fasciculus retroflexus to the interpeduncular nucleus are entirely from the medial habenula.

This limited course of axons from the medial nucleus has also been shown by Nauta (cat, '58) with the Nauta-Gygax technique.

The normal fibers seen in the compact portion may be accounted for by fibers from the stria to the parafascicular

area, or efferents to the habenula from midbrain areas. Guillery (cat, '59) has described fibers originating from or coursing through the ventral tegmental area of Tsai, that ascend in the rostral part of the tractus habenulopeduncularis (fasciculus retroflexus of Meynert). Massopust and Thompson (rat, cat, '62) have described an interpedunculo-diencephalic tract ascending in the tractus habenulo-peduncularis, some fibers terminating in the dorsolateral and dorsomedial thalamic nuclei and lateral habenula.

Efferents from the lateral habenula form a diffuse lateral pathway and correspond to the "habenulo-tegmental tract" described by Bürgi and Bucher (1955), and also by Nauta (1958), in the cat. Bürgi has used the Marchi method, Nauta the Nauta-Gygax and their observations have been confirmed by the present studies using the Fink-Heimer technique. This diffuse pathway in the cat rejoins the fasciculus retroflexus after a course through the centre median nucleus and mesencephalic reticular formation (present work, Fig. 10). Knook was unable to demonstrate the existence of this pathway which might indicate a species difference.

Degeneration from this pathway can be followed beyond the interpeduncular nucleus to the upper midbrain, terminating in the central as well as in the dorsal tegmental nuclei of Von Gudden, (Nauta, '58, Knook, '65, present studies, '68). The course and termination of these very fine fibers was difficult to see in Nauta stained material. In contrast, the

Fink-Heimer technique gave a very clear picture of terminals and the degenerated fibers appeared darker and greater in number.

Adey et al (1956) has described a projection from the entorhinal area, in the phalanger, which follows the stria medullaris and distributes to dorsolateral regions of the mid-brain tegmentum without synapsing in the habenula. In the cat, a comparable pathway could not be identified, (Nauta and present studies) and although a large lesion involved the stria medullaris, fiber degeneration beyond the parafascicular area was still absent. Again, this discrepancy may be due to species variation.

This lesion in the stria medullaris shows massive degeneration in the parafascicular area, indicating an origin other than or in addition to the habenula. A possible source of these afferents may be the dorsomedial nucleus of the thalamus since a portion of the anterior dorsomedial nucleus was also lesioned. Johnson (cat, '61), could trace reciprocal efferent fibers from the nucleus dorsomedialis into the nucleus centromedianum but he did not describe any fibers coursing in the stria medullaris. The origin of these fibers to the para-fascicular area cannot be defined with certainty as coming exclusively from the habenula.

Habenulo-tectal fibers cannot be determined from this material because most lesions damaged tectal areas. These fibers are more easily seen in sagittal sections and might be missed in a frontal plane.

Cragg (rabbit, '61a), after a lesion of the nucleus habenularis lateralis, has described degenerated fibers crossing in the habenular commissure to terminate in the corresponding part of the contralateral nucleus habenularis. In the present study, degenerated fibers are found in the contralateral habenula and stria medullaris. However, degeneration is not seen contralateral to the site of the lesion or in the interhabenular commissure. Because a lesion of the lateral habenula necessarily interrupts stria medullaris fibers no firm statement can be made regarding commissural fibers from the lateral habenula.

Miline et al (1965) have reported some experiments which suggest the existence of a functional connection between the habenulo-pineal complex and the hypophyseo-thyroidal axis. Fibers have been reported to reach the epiphysis from the habenular commissure. These fibers were thought to arise from the striae medullares whereas other fibers would originate from nerve cells in the habenular nuclei and in the commissure. It is the opinion of Ariëns Kappers ('65), that habenular fibers reaching the pineal are exclusively aberrant commissural fibers derived from the habenular commissure. This view is supported by Kenny ('67) who states that "fibers from the habenular and the posterior commissure do not stand in functional relationship with the pineal body".

In this experiment, degenerating fibers are seen in the dorsal portion of the habenular commissure but no fibers are seen in the pineal organ.

CHAPTER V

SUMMARY

1. The cells of the medial habenular nucleus are of Type 1 characterized by a small, round cell body and short multi-branching dendrites.

   The cells of the lateral nucleus are of type 2 and 3. Type 2 is fusiform is shape with radiate dendritic branches occasionally with 'spines'. This is the only type found to have spine-like projections on the perikaryon.

   Type 3 is pyramidal in shape with diffusely branching dendrites also possessing spines.

2. The axons of these neurons are studied by means of experimental lesions in the habenula and the sections then stained by the Nauta and Fink-Heimer technique. The use of these newer methods allows a more precise description of their sites of termination.

3. The medial nucleus projects to the ventral tegmental area of Tsai and the interpeduncular nucleus via the compact fasciculus retroflexus.

4. The lateral nucleus projects to the ventral tegmental area via a diffuse pathway through the centre-median nucleus and mesencephalic reticular formation. Longer

fibers continue dorsally into the midline raphe of the midbrain distributing terminal fibers to the predorsal area, tegmental nuclei of Von Gudden and caudal central grey.

5. Fibers from the stria medullaris cross in the interhabenular commissure and course rostrally in the contralateral stria. Degenerating terminals are seen in the contralateral, lateral habenula.

6. These observations are in general agreement with the anatomical studies of earlier investigators.

FOOTNOTES

1. Cajal, p. 66.
2. Cajal, pp. 67-68.
3. Ramon-Moliner, pp. 215-216.
4. Ramon-Moliner, p. 226.
5. Marburg, pp. 221-222.
6. Cragg, pp. 406-407.
7. Gamble, '52, p. 193.
8. Nauta, '58, pp. 27-28.
9. As used in this text, the dendritic trunk will be the same as the primary branch, with branches from this stem the secondary branch and so on.

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